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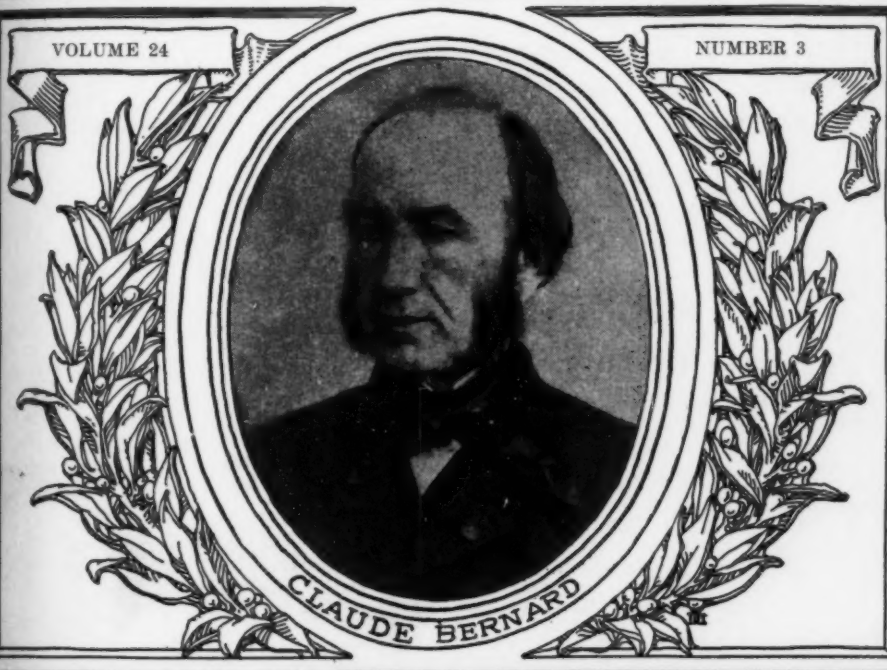

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THE ABSORPTION AND RETENTION OF CAROTENE AND VITAMIN A BY HENS ON NORMAL AND LOW FAT RATIONS [1]

WALTER C. RUSSELL, M. WIGHT TAYLOR, HARRY A. WALKER AND LOUIS J. POLSKIN

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ONE FIGURE

(Received for publication February 7, 1942)

The efficiency of absorption and utilization of carotene and of vitamin A under various conditions has been the subject of several investigations. Ahmad ('31) found that carotene from either raw carrots or cooked spinach was absorbed nearly twice as well by humans when the diet contained fat as when the fat was omitted. That fat aids in the absorption of carotene was also found by van Eekelen and Pannevis ('38). Basu ('37) claimed that fat is essential for the absorption by rats of both carotene and vitamin A. On the other hand, De ('37) and Wilson, Das Gupta and Ahmad ('37); working with rats and humans, respectively, found no difference between the absorption of vitamin A on diets containing high and low levels of fat. Gray, Hickman and Brown ('40) reported that rats stored in the liver nearly six times as much vitamin A from cod liver oil as from β-carotene. Very little work has been done using the hen as an experimental animal.

Record, Bethke and Wilder ('37) and Peterson, Hughes and Payne ('39) found no difference between the absorption of carotene and of vitamin A by hens fed normal diets. A recent

[1] Journal series paper of the New Jersey Agricultural Experiment Station, Rutgers University, Department of Agricultural Biochemistry.

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communication from this laboratory (Russell, Taylor and Polskin, '40) reported that chicks fed a diet containing less than 0.1% fat were able to utilize sufficient crystalline carotene to maintain normal growth for 14 weeks. However, the carotene was fed at a level approximately five times the minimum requirement, since these studies were concerned mainly with fat requirements. In the present work attention is given primarily to the effect of normal and low fat diets on the absorption and retention of carotene and vitamin A.

EXPERIMENTAL

White Leghorn hens were housed in individual pens, with wire mesh bottoms, in a fattening battery and fed an all-mash ration of the following composition: white corn meal 20, wheat flour middlings 20, wheat bran 20, pulverized whole oats 20, meat scrap (55% protein) 10, skimmed milk powder 5, ether-extracted alfalfa leaf meal 5, calcite flour 2, and sodium chloride 1. This constituted the normal ration. The alfalfa was extracted with ether in order to reduce as much as possible the carotene content of the ration. The low fat ration was prepared by extracting the normal ration in percolators at room temperature with a mixture of 3 parts diethyl ether and 1 part 95% ethyl alcohol. As determined by 16 hours extraction with diethyl ether in a Soxhlet extractor, the fat content was 0.07%. Sucrose was added to the low fat diet at a 4% level to replace the fat which had been removed. Two per cent of yeast [2] washed with several changes of ether, was added to both rations to ensure an adequate supply of the vitamin B complex. All birds received 10 minutes of ultraviolet radiation from a quartz-mercury vapor lamp three times a week, the distance from the burner to the floor of the cage being about 60 cm.

The carotene and vitamin A supplements were fed daily by capsule. The factors were dissolved in diethyl ether, an appropriate amount pipetted into capsules, and the solvent evaporated. The beginning and end of each period was identified

[2] Northwestern Yeast Powder.

by feeding carmine or charcoal and the last vitamin feeding was given at least 20 hours before the end of a period. The excreta were collected daily, put into 95% ethyl alcohol, and stored in a refrigerator (40°C.) until analyses could be made. Tests showed that the factors were stable under these conditions. Carotene was determined in the food and in the droppings by the method of Peterson, Hughes and Freeman ('37), the final solutions being read with a Fisher Electrophotometer using a Corning no. 430 filter. Vitamin A was determined by the Carr-Price reaction ('26), the readings being made at 615 mμ with a Bausch and Lomb Universal Spectrophotometer.

FEEDING STUDIES WITH CAROTENE

White Leghorn hens were maintained without added carotene on the normal and low fat rations for a depletion period of 10 weeks, during which time feed consumption was measured and droppings collected in periods 1, 2, and 3, and for the normal ration group in period 4, as shown in table 1. In period 4, which began at the end of the depletion period, a quantity of crystalline carotene, calculated to be equal to that consumed in the normal ration, was fed to the birds on the low-fat ration. In periods 5 and 6, which followed immediately, increased amounts of carotene were fed to both groups. Attempts were made in periods 4, 5, and 6, to equalize the carotene intake of the two groups.

Results and discussion

In period 1 (table 1) it was noted that the total yellow pigment excreted by hens on the low fat ration, expressed as carotene, was greater than the quantity of carotene consumed. To determine the nature of the pigment in the extract, absorption curves were obtained by means of a Bausch and Lomb Universal Spectrophotometer and compared with curves for both purified carotene and an extract of excreta from the normal group. The results showed that the predominant pigment in the excreta of the low fat group was not carotene (fig. 1), whereas the extract from the normal group showed

a curve characteristic of this substance. Since the pigment was predominantly not carotene, and it was assumed that hens on the normal ration would excrete at least as much of the unknown pigment as those of the low fat group, it seemed advisable to apply at least a minimum correction to the values obtained with the normal ration. The quantities deducted in

TABLE 1

Daily absorption of carotene by the hen on normal and low fat rations.

PERIOD NO.[1]	NO. OF HENS	DAYS ON EXPT.	FOOD CONSUMPTION	CAROTENE CONSUMED			TOTAL YELLOW PIGMENT EXCRETED EXPRESSED AS CAROTENE	CAROTENE EXCRETED	CAROTENE ABSORBED	
				In feed	By capsule	Total				
			gm.	μg.	μg.	μg.	μg.	μg.	μg.	%
1 N[2]	4	0	58.8	60	0	60	38	26	34	57
LF	12		78.4	8	0	8	12	0	8	100
2 N	2	11	44.1	45	0	45	20	15	30	67
LF	2		49.0	5	0	5	5	0	5	100
3 N	8	43	62.7	64	0	64	32	28	36	56
LF	8		58.8	6	0	6	4	0	6	100
4 N	4	71	75.5	77	0	77	44	40	37	48
LF	4		78.4	8	58	66	47	43	23	35
5 N	4	74	72.6	74	67	141	58	54	87	61
LF	4		78.4	8	117	125	99	95	30	24
6 N	4	79	69.6	71	200	271	92	88	183	67
LF	4		78.4	8	232	240	205	201	39	16

[1] Periods 1, 4, 5 and 6 were each 3 days and periods 2 and 3 were 4 days in length.

[2] N — Normal ration: 3.83% fat; 102 μg. carotene per 100 gm.; LF — Low fat ration: 0.07% fat; 10 μg. carotene per 100 gm.

periods 1, 2, and 3 were those observed on the low fat ration during the corresponding periods. It was assumed that in period 3, excretion of the non-carotene pigment had reached a minimum and therefore this value, 4 μg., was subtracted in periods 4, 5, and 6. Kemmerer and Fraps ('38) reported the presence of a non-carotenoid pigment of unknown character in the excrement of rats and chickens receiving feeds containing negligible amounts of carotene. Presumably the pigment found in the present investigation was the same as

that reported by Kemmerer and Fraps but no attempt was made to determine its nature.

The absorption of carotene on the normal ration at the beginning of the experiment was 57% and for period 4, after 71 days on the ration, 48%. Except for period 2, in which observations were made with only two hens, the percentage absorption tended to decrease as the experiment progressed. In the case of hens on the low fat feed, carotene was not excreted during the first 3 periods as shown by spectrophoto-

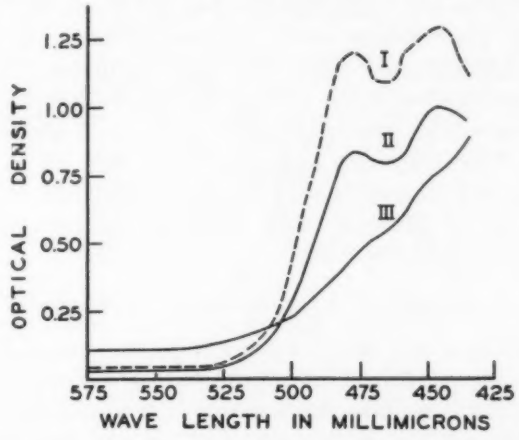

Fig. 1 Absorption curves for purified carotene (I), for the extract from excreta of birds receiving carotene (II), and for the yellow coloring matter from excreta of birds fed practically no carotene (III).

metric data and therefore it was concluded that the small quantity of carotene in the feed, assuming no destruction in the tract, was absorbed.

When the carotene intake was increased by feeding crystalline carotene by capsule so that essentially the same quantity was consumed by each group, the hens on the normal ration showed an increasing tendency to absorb this factor. The quantity of carotene absorbed was definitely less on the low fat ration than on the normal. Although the quantity absorbed increased with increasing dosage, there was a pro-

gressive decrease in the percentage absorbed. Thus, when the intake on the low fat ration was increased 3.5 times, the quantity absorbed increased only 1.7 times while under the same conditions on the normal ration, the increase was fivefold. Since the fat content of the ration is the differentiating factor, it is concluded that fat is necessary in the poultry ration for satisfactory absorption of carotene. The quantity of carotene absorbed may be limited by the quantity of fat present and it is possible that in period 6, when 67% of the carotene fed in the normal ration was absorbed, the birds may have been approaching maximum absorption. On the other hand, Kemmerer and Fraps ('38) reported that as the quantity of carotene supplied in the form of dehydrated alfalfa leaf meal was increased in the ration, the percentage absorption decreased. The difference between the results of the two investigations is probably due to the availability of carotene for absorption, the crystalline form, in the presence of fat, being more readily available.

INJECTION STUDIES WITH CAROTENE

The question was raised as to whether any of the absorbed carotene appeared in the excreta. In an attempt to answer this question, injection studies were conducted parallel with the feeding studies of periods 4, 5, and 6. The birds used were from the same group as in the previous studies and had the same preliminary period on the normal and low fat diets. A carotene suspension in water made according to the methods described by Fodor and Schoenfeld ('31) was injected daily, intravenously, in four birds in both the normal and low fat groups. An attempt was made to inject essentially the same quantity of carotene as was fed daily by capsule during periods 4, 5, and 6, although this was not possible in every case. Also, each group was given approximately the same level of carotene according to the average feed consumption as determined during the depletion period. The ration, experimental procedure, and analytical methods were the same as those in the preceding part of this investigation.

Results and discussion

As will be seen in table 2, when as much as 195 μg. of carotene was injected intravenously, daily, in the low fat birds, none was excreted. The carotene excreted by the hens on the normal ration for periods 8 and 9, based on the amount consumed in the feed but not on the additional amount which was injected, was 32% and 36%, respectively. These values are

TABLE 2

Daily retention of carotene injected intravenously in hens on normal and low fat rations.

PERIOD NO.[1]	DAYS ON EXPT.	FOOD CONSUMPTION	CAROTENE ADMINISTERED			YELLOW PIGMENT EXCRETED	CAROTENE EXCRETED	
			In feed	Injected	Total			
		gm.	μg.	μg.	μg.	μg.	μg.	%[2]
7 N[3]	71	53.9	55	0	55	27	22	40
LF	71	68.6	7	49	56	5	0	0
8 N	74	46.1	47	53	100	20	15	32
LF	74	58.8	6	98	104	5	0	0
9 N	79	56.9	58	180	238	27	21	36
LF	79	68.6	7	195	202	6	0	0

[1] The collection periods were 3 days in length. Each value is an average of the results from 4 hens.

[2] Calculated on the basis of the carotene in the feed.

[3] N — Normal ration: 3.83% fat. LF — Low fat ration: 0.07% fat.

lower than those obtained in periods 1 to 4 inclusive which, by calculation from the absorption values in table 1, ranged from 33% to 52%. In period 9-N the excretion of the pigment was 36% of that in the feed, despite the daily injection of 180 μg. of carotene. It is believed therefore that the carotene which appears in the excreta is that which has escaped absorption and not that excreted into the tract.

VITAMIN A STUDIES

Experimental

Studies of the absorption and retention of vitamin A by hens fed normal and low fat diets were carried out in much the same manner as in the case of carotene. The birds under-

went a preliminary depletion period which varied from 2 to 24 weeks, the variation in time being due to the addition of new birds to an older group which had been used in a previous experiment. Vitamin A ester was fed by capsule in increasing amounts during 13 successive 3-day periods and the quantity which appeared in the droppings was determined. The ester was a molecular distilled concentrate having a potency of 200,000 U.S.P. units per gram and was dissolved in ether for the filling of capsules.

For 1 month after the last feeding of the vitamin the hens were continued on the two rations. At the end of this period they were sacrificed and the vitamin A content of the livers determined.

Results and discussion

During the first 7 periods (table 3) vitamin A was increased progressively from 375 units in period 2, to 8250 units in period 7, a total of 18,850 units being fed during these periods. Despite this large intake of vitamin A, only two birds in the normal and one in the low fat group showed what was interpreted to be traces of the factor in the droppings. When an additional 12,500 units was fed in period 8, making a total to the end of that period of 31,350 units, the excreta of two of the normal and one of the low fat birds contained the factor. After an additional 18,300 units was fed in period 9, making a grand total of 49,650 units, vitamin A appeared definitely in the excreta of all members of the two groups except one, no. 1043. A small amount of vitamin A appeared in the excreta of this hen when an additional 24,800 units was fed in period 10. It is of interest to note that throughout the experiment this individual excreted the lowest percentage of vitamin A intake of any of the birds.

The fact that vitamin A was not detected during the first 7 periods, does not necessarily mean that none was excreted. If it is assumed that the hens failed to absorb the same percentage of the vitamin during these periods as later in the experiment, the calculated amounts which would have been

present in the droppings of these early periods should have been very small. These amounts could not be measured by the technique employed, partly because of a small error inherent in reading the spectrophotometer but more because of the fleeting nature of the color developed and the presence of a relatively high level of non-vitamin A chromogens which produced a somewhat variable general absorption.

TABLE 3

Excretion of vitamin A by hens on normal and low fat rations — 3-day periods.

PERIOD NO.	VITAMIN A ESTER FED DURING A 3-DAY PERIOD U.S.P. XI UNITS	PER CENT OF VITAMIN A EXCRETED						
		NORMAL GROUP				LOW FAT GROUP		
		Bird No. 1022	Bird No. 1316	Bird No. 271	Bird No. 651	Bird No. 833	Bird No. 1028	Bird No. 1043
	Weeks of depletion →	24	3	2	3	8	24	24
1	0	0	0	0	0	0	0	0
2	375	0	0	0	0	0	0	0
3	700	0	0	0	0	0	0	0
4	1400	0	0	0	0	0	0	0
5	2650	0	0	0	0	0	0	0
6	5475	0	0	0	0	0	0	0
7	8250	trace	0	0	trace	0	trace	0
8	12,500	16.5	trace	trace	10.9	trace	6.8	0
9	18,300	14.8	7.9	8.3	10.0	5.6	8.3	trace
10	24,800	15.6	6.9	5.4	9.3	4.7	4.9	2.9
11	38,500	17.5	6.9	6.1	25.6	4.4	7.8	3.3
12	46,200	12.1	6.0	6.0	19.3	3.9	8.7	4.8
13	57,000	12.2	9.1	7.8	12.0	4.4	5.3	3.8
Average excretion %		14.8	7.4	6.7	14.5	4.6	7.0	3.7
Average retention %		85.2	92.6	93.3	85.5	95.4	93.0	96.3

The percentage of vitamin A excreted appeared to be characteristic of each hen and remained of the same order for each throughout the experiment, although, of course, the actual quantity excreted increased with increasing dosage. Furthermore the length of the depletion period, whether 2 or 24 weeks, had no effect on either the percentage of vitamin A excreted or the first appearance of a detectable amount of the vitamin. It was first detected in the droppings of those birds which excreted the highest percentages. Thus, two of the first three birds to eliminate vitamin A had been on the depletion diet

for 24 weeks, and one, no. 1022, showed the highest percentage excretion of the group. These data indicate that any measurement of the efficiency of absorption of vitamin A would be of no value in determining the nutritional status of the hen. It is of interest to note that even after 24 weeks' depletion there were no gross symptoms of vitamin A deficiency and that the body weights of the birds did not change appreciably during the experiment.

Twenty-two hours after the close of the final period, vitamin A could not be detected in the excreta of either group and therefore it is concluded that the excretion for each period is essentially from the quantity of the vitamin fed during that period. The same observation was made 3 days later and therefore it is concluded that the vitamin is not eliminated from the body stores either through the kidneys or the intestine.

Two of the low fat birds showed a higher percentage retention than did any of the four birds of the normal group and the average excretion of the low fat group was approximately half that of the normal birds. It is probable that this is not a significant difference because of the small number of individuals involved.

Each 200 units of vitamin A ester fed was contained in approximately 1 mg. of oil solvent. Later experiments with growing chicks (Polskin, '40), in which was studied the effect of fat on the absorption of crystalline carotene and of vitamin A alcohol, indicate that more of these factors reach the liver when fed with fat. In these preliminary studies 1 mg. of fat (corn oil) was administered with each 200 units of vitamin A (or carotene) as in the feeding of vitamin A ester in the present experiment, and the fat contents of the rations were essentially the same in both experiments. The total fat intake was raised slightly though probably not significantly by the supplements. In period 7, for example, in which traces of vitamin A first appeared in the droppings, the quantity of fat fed as the solvent raised the level of fat in the diet from 0.07 to 0.08%. Therefore, in the present experiment, the fatty

solvent of the vitamin A ester probably favored absorption of the factor, either by a protective role in the tract or by actually aiding in absorption, but the extent of its influence is not known.

The results on both the low fat and the normal rations show that the vitamin A ester is absorbed more completely than is carotene and suggest that vitamin A values calculated from the carotene content of the ration or based on rat assays may not be entirely reliable for the hen.

TABLE 4

Vitamin A content of livers of hens on normal and low fat rations 1 month after receiving large doses of vitamin A.

GROUP	BIRD NO.	WT. OF LIVER	VITAMIN A PER GM. OF LIVER U.S.P. XI	TOTAL VITAMIN A IN LIVER U.S.P. XI	VITAMIN A RECOVERED IN LIVER
		gm.	*units*	*units*	%
Normal	1316	15.1	6875	94,380	43.7
	271	19.6	4650	91,140	42.4
	1022	19.0	3650	69,350	32.1
	651	53.7	1212	65,120	30.1
Average			4096	80,000	37.0
Low fat	1028	21.3	630	13,425	6.2
	833	20.0	490	9,850	4.5
	1043	20.3	420	8,525	3.9
Average			513	10,600	4.9

The hens were maintained on the respective rations for 1 month after the last dose of vitamin A was administered, after which they were killed and the quantity of the factor in the liver was determined. In table 4, it is noted that in the normal group the average vitamin A in the liver was 80,000 units, whereas in the low fat group, it was 10,000 units. The former value is 37% of the total number of units administered in the 13 periods and the latter only 4.9%. Since there was no difference between the normal and low fat group in their vitamin A excretions it was assumed that the liver storage of the factor was the same in both groups. This was not determined on the hens because of the small number available but in another experiment with growing chickens (Polskin, '40)

it was found that the liver content of vitamin A, following a feeding regime similar to that of the present experiment, was essentially the same on both normal and low fat rations. Therefore, assuming equal storage, it must be concluded that the presence of a normal amount of fat in the ration was accompanied by a much greater retention of vitamin A during the depletion period when no vitamin A was fed, than occurred in the case of the low fat ration.

SUMMARY AND CONCLUSION

1. In hens the absorption of carotene in the crystalline form is improved by the presence of fat in the ration.

2. On the low fat ration, and presumably on the normal, there appears in the excreta a yellow pigment which has the solubility properties of carotene, but which, according to spectrophotometric determination, is not a member of the carotene group of pigments.

3. Neither carotene nor vitamin A is eliminated from the body stores by way of either the kidney or the intestine.

4. Absorption of vitamin A by hens was essentially the same on normal and low fat rations. The length of the depletion period, prior to feeding vitamin A, had no effect upon the percentage of the factor absorbed. As the feeding levels were increased, the percentage of the factor excreted remained essentially the same in both groups and was characteristic of the individual hen. The presence of a small quantity of fat as the vehicle for vitamin A ester (1 mg. per 200 units) may have favored absorption.

5. The retention of vitamin A in the liver was greater in the hens which received the ration with normal fat content.

LITERATURE CITED

AHMAD, B. 1931 The fate of carotene after absorption in the animal organism. Biochem. J., vol. 25, p. 1195.

BASU, N. K. 1937 Vitamin A and fat metabolism. Zeitschr. fur Vitaminforsch., vol. 6, p. 106.

CARR. F. H., AND E. A. PRICE 1926 Color reaction attributed to vitamin A. Biochem. J., vol. 20, p. 497.

DE, N. K. 1937 The assimilation of vitamin A and carotene by rats from some common foods with a note on the conversion factor proposed by the International Vitamin Conference. Ind. J. Med. Res., vol. 24, p. 751.

FODOR, A., AND R. SCHOENFELD 1931 Darstellung und Eigenschaften wässeriger Carotinlösungen. Biochem. Z., vol. 233, p. 243.

GRAY, E. L., K. C. D. HICKMAN AND E. F. BROWN 1940 The state of vitamin A in the liver of the rat after feeding various forms of the vitamin. J. Nutrition, vol. 19, p. 39.

KEMMERER, A. R., AND G. S. FRAPS 1938 The digestibility of carotene by rats and chickens. J. Nutrition, vol. 16, p. 309.

PETERSON, W. J., J. S. HUGHES AND H. F. FREEMAN 1937 Determination of carotene in forage. Ind. Eng. Chem., Anal. Ed., vol. 9, p. 71.

PETERSON, W. J., J. S. HUGHES AND L. F. PAYNE 1939 The carotenoid pigments. Occurrence, properties, methods of determination, and metabolism by the hen. Kansas Agr. Exp. Sta., Tech. Bull. 46, 74 pp.

POLSKIN, L. J. 1940 Studies on the absorption and retention of vitamin A and carotene by the chicken on normal and low fat rations. Doctor's Thesis, Rutgers University, New Brunswick, New Jersey.

RECORD, P. R., R. M. BETHKE AND O. H. M. WILDER 1937 The vitamin A requirements of chicks with observations on the comparative efficiency of carotene and vitamin A. Poultry Sci., vol. 16, p. 25.

RUSSELL, W. C., M. W. TAYLOR AND L. J. POLSKIN 1940 Fat requirements of the growing chick. J. Nutrition, vol. 19, p. 555.

VAN EEKELEN, M., AND W. PANNEVIS 1938 Absorption of carotenoids from the human intestine. Nature, vol. 141, p. 203.

WILSON, H. E. C., S. M. DAS GUPTA AND B. AHMAD 1937 The absorption of carotene and vitamin A in the human subject. Ind. J. Med. Res., vol. 24, p. 807.

THE EFFECTIVENESS OF LINOLEIC, ARACHIDONIC, AND LINOLENIC ACIDS IN REPRODUCTION AND LACTATION [1, 2]

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In comparing individual fatty acids or their derivatives for biological activity, restoration of growth and prevention or cure of dermal lesions have served heretofore as the criteria (Burr and Burr, '30; Burr, Burr and Miller, '31; Turpeinen, '38; Hume et al., '40). Although reproduction is seriously impaired in fat deficiency, little is known concerning the unsaturated acid requirements for this important biological function. Normal litters of rats have been produced with dietary supplements of natural fats such as lard and butterfat (Burr and Burr, '30; Evans et al., '34; Maeder, '37), and inferior litters with linoleic acid either as a concentrate (Evans et al., '34), or as the pure methyl ester (Mackenzie et al., '39). Data concerning other acids or esters are lacking.

In experiments with low-fat diets most workers have used ether-extracted yeast as the source of the vitamin B complex (Burr and Burr, '30; Evans et al., '34). The use of this material is open to criticism since it has been shown that yeast contains from 2.5 to 4% of lipids which are firmly bound and non-extractable by ether (Smedley-Maclean, '22; Newman and Anderson, '33). Mackenzie, Mackenzie and McCollum

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('39) have recently used a water-soluble extract from yeast, but unfortunately, they fed a relatively low level of methyl linolate.

Recent work by Schneider and Steenbock ('39) had shown that a commercially available water-soluble rice bran concentrate very low in lipids, constituted a good source of the vitamin B complex when fed in a diet which contained egg white as the source of protein. In the present work this product was fortified with riboflavin to make a satisfactory diet in which purified casein served as the protein. In parallel experiments using this diet of extremely low fat content and a diet containing ether extracted yeast, unsaturated acids of high purity were compared for their nutritive value.

EXPERIMENTAL

Diets

The first diet, designated the "Rice-Extract Diet," contained glucose [3] and a rice bran concentrate [4] with added riboflavin; the second, which was a duplication of the Burr and Burr diet, designated the "Yeast Diet," contained sucrose and ether-extracted yeast (table 1).

Both diets were supplemented with 100 μg. carotene, 35 μg. calciferol, 350 μg. tocopherol [5] and 100 μg. vitamin K_1 [5] per rat per week. These supplements were dissolved in a liquid fraction of hydrogenated coconut oil (I. V. = 0.1), and 1 drop of the solution was administered twice weekly. The flavin was dissolved in N/50 acetic acid, mixed with the rice bran concentrate and incorporated in the ration. The yeast was fed in small dishes.

Although the yeast had been extracted with ethyl ether in a continuous extractor for 3 days, the daily intake of unsaturated lipid from yeast was many times as high as that from the rice bran concentrate (table 2). This was revealed

[3] Cerelose.

[4] Vitab Type II, National Oil Products Company.

[5] We are indebted to Merck and Company, Inc. for the α-tocopherol and vitamin K_1.

by treatment of both desiccated and moist samples of the two materials with acidified alcohol and subsequent extraction of the products. In the first case 100 gm. of yeast or rice bran concentrate were dried over P_2O_5 and refluxed for 2 hours

TABLE 1

Composition of diets.

	RICE-EXTRACT DIET	YEAST DIET
Casein (alcohol-extracted)[1]	18	18
Wesson salts	4	4
Cerelose	75	..
Sucrose	..	72
Ether-extracted yeast [2]	..	0.65 gm./day
Rice bran concentrate [3] + 2 mg. riboflavin/kg. ration	3	..

[1] Labor for the purification of the casein was furnished by the Works Progress Administration.

[2] Brewers yeast, Anheuser-Busch Company.

[3] Vitab type II, National Oil Products Company.

TABLE 2

Lipid content of dietary constituents by cleavage with HCl.

CONSTITUENT	TOTAL LIPID (P. E. EXTRACT)	IODINE VALUE	APPPROXIMATE DAILY LIPID INTAKE [1]	
			Total	Unsaturated (calcd. as linoleic)
	%		*mg.*	*mg.*
Yeast (desiccated)	2.43	85.7	16.0	7.6
Rice bran concentrate (desiccated)	0.36	64.0	1.1	0.4
Yeast (moist)	4.21	84.7	27.0	12.6
Rice bran concentrate (moist)	0.44	41.6	1.3	0.3
Casein	0.11	40.4	2.0	0.4

[1] Based on an assumed consumption of 10 gm. of diet daily.

with 300 cc. of a solution of 3% dry HCl in absolute alcohol. The extract was diluted with an equal volume of water and shaken with three 100 cc. portions of chloroform. The alcohol-insoluble residue was extracted with chloroform for 2 days

in a Soxhlet. The chloroform extracts were combined, washed thoroughly with water, dried over sodium sulphate and freed from chloroform under reduced pressure. The residue was taken up in 5 to 10 cc. of chloroform and freed from non-lipid substances by precipitation with petroleum ether. The petroleum ether-soluble material was washed with water, dried and freed from solvent.

Hydrolysis with 3% HCl in 80% alcohol produced even a larger yield of lipid from the yeast. The procedure was identical with that detailed above except that before refluxing, 20% of water was added to the yeast to make it equivalent in water content to the rice bran concentrate.

The casein was purified by extraction with warm alcohol (50°C.). Nevertheless, it yielded a small amount of lipid on acid hydrolysis (table 2). For the hydrolysis 100 gm. were heated with 300 cc. of dilute HCl (1:3) for 20 hours on a steam bath. The solution was cooled, treated with an additional 25 cc. of HCl and extracted with three 100 cc. portions of redistilled petroleum ether. The combined extracts were washed with three 100 cc. portions of water and dried over sodium sulphate. The solvent was removed under reduced pressure and the residue weighed, giving a yield of 90 mg. When the aqueous phase was refluxed vigorously for 6 hours more, an additional 20 mg. of lipid were obtained giving a total of 110 mg.

Based on these analyses, the yeast furnished 27 mg. of lipid per rat daily. Assuming an average food intake of 10 gm. daily the rice bran concentrate furnished 1.3 mg. of lipid. In terms of unsaturated lipid these differences were even greater. Expressing the unsaturated compounds in terms of linoleic acid, a maximum of 12.6 mg. of linoleic acid was furnished by the yeast and 0.4 mg. by the rice bran concentrate. Adding the lipid which was supplied by the casein these values become 13.0 and 0.8 mg. for the yeast and rice-extract diets, respectively.

The fatty acids were fed as the ethyl esters. The ethyl linolate and linolenate were prepared from corn oil and

linseed oil by a modification of the Rollett method ('09). The ethyl arachidonate was prepared from beef suprarenal phosphatide [6] by low temperature crystallization and fractional distillation (Shinowara and Brown, '40). The distilled ethyl esters had the following iodine values (Wijs, 4 hr.): ethyl linolate 166 (theor. 164.8); ethyl linolenate 245 (theor. 248.9); ethyl arachidonate 299 (theor. 305.5).

Preparation of animals

To minimize the pre-experimental storage of essential unsaturated fatty acids, litters at 12 days of age were transferred with their mothers to a diet consisting largely of potatoes (Quackenbush et al., '39). The young were weaned when they weighed 40 gm. and placed in individual metal cages where they consumed the experimental diets ad libitum. Thirty female rats were given the rice-extract diet, and twelve females and fourteen males the yeast diet. After 12 weeks the females on each diet were divided into five groups. The heaviest animals, weighing about 178 gm., received only the basal diet; the lightest, which averaged 131 gm., received in addition supplements of cottonseed oil. The remainder were divided equally among the other groups. Three weeks later all were mated with normal males. After the finding of sperm in the vaginal smear the allowances of fat-soluble vitamins and vitamin B complex were doubled. The litters were reduced to six young each, 24 hours after parturition. During lactation the dose of the vitamin B complex was tripled.

RESULTS

Growth and general appearance

Animals fed the low-fat diets grew to maturity, but at the twelfth week their body weights were significantly less than those of comparable animals fed our stock diet (Steenbock, '23). Those getting the rice-extract diet weighed 151 gm. and

[6] We are indebted to Dr. O. Kamm of Parke-Davis and Company for this preparation.

those receiving the yeast diet 154 gm., while females in the stock colony weighed 195 gm. at the same age.

The addition of various lipid supplements after 12 weeks on either of the two basal diets resulted in growth, the magnitude of which depended upon the nature of the supplement as well as on the basal ration. In 3 weeks cottonseed oil produced a gain of 50 gm. in the animals ingesting the rice-extract diet and 30 gm. in those fed the yeast diet. Ethyl linolate produced a gain of 26 gm. on either diet, while ethyl arachidonate produced a gain of 34 gm. on the rice-extract diet and 19 gm. on the yeast diet. Ethyl linolenate produced much smaller gains, viz., 14 gm. on the rice-extract diet and only 8 gm. on the yeast diet. Without supplements the animals on the former diet gained slightly, viz., 7 gm.; those on the latter diet did not gain. A further demonstration of the necessity of fatty acids for growth was obtained from two groups of animals which had received ethyl linolate or cottonseed oil beginning with the second instead of the twelfth week on the rice-extract diet. Those receiving ethyl linolate weighed 188 gm. and those getting cottonseed oil weighed 195 gm. at the end of the twelfth week.

The animals on either of the low-fat diets developed a scaliness of the hind paws and tail after they had been on the experimental diet from 9 to 12 weeks. No scaliness or loss of hair around the eyes, nose or mouth was noted. Within 3 weeks after supplements of cottonseed oil, ethyl linolate or ethyl arachidonate were given, the scaliness of the hind paws and tail was cured completely. The cured animals were sleek and in every way appeared like stock rats. However, with ethyl linolenate the scaly condition of the hind paws and tail persisted even after 7 weeks.

Reproduction and lactation

A failure of normal parturition always ensued on the basal diets (table 3). Labor began at term, but parturition was accompanied by excessive hemorrhage and was not completed for 2 to 3 days. The animals lost weight and became extremely

weak and anemic. Two of the females died before giving birth to their young. All of the young died within 48 hours after birth.

When supplements of cottonseed oil, ethyl linolate, or ethyl arachidonate were fed, the needs of both parturition and lactation were met. The rice-extract diet gave approximately the same results as the yeast diet with the same fat supple-

TABLE 3

The effects of fatty acid esters on reproduction.

GROUP	SUPPLEMENT	AMOUNT FED DAILY	RICE-EXTRACT DIET					YEAST DIET	
			No. of rats	Gestation period	Mean no. young per litter	Born alive	Weaned	Born alive	Weaned
	12th to 19th week:	*drops*		*days*		%	%	%	%
1	None	..	6	25	4	8	0	73	0
2	Ethyl linolate	5	5	22	6	100	34	100	83
3	Ethyl linolenate	5	5	24	3	56	0	83	0
4	Ethyl arachidonate	5	5	22	9	100	67	100	55
5	Cottonseed oil	10	2	22	5	100	80	100	92
	2nd to 19th week:								
6	Ethyl linolate	5	5	22	6	100	79	..	..
7	Cottonseed oil	5% in diet	2	22	6	100	89	..	..

ments. When ethyl linolate was given after the second week of the experimental period, the results compared favorably with those obtained on our stock diet.

By way of contrast ethyl linolenate produced results only slightly better than those obtained on the basal diet. While the gestation period was prolonged and vaginal bleeding was the same as on the basal diets, three of the fourteen young born alive in group 3, survived the first week. One of these reached a weight of 40 gm. at 5 weeks and the other two a weight of 31 gm. at 4 weeks of age, but all died shortly thereafter. All had scaly feet and tails, in contrast with the young receiving the other lipid supplements, which were entirely normal.

Other fat deficiency symptoms

Failure of oestrus as reported by Burr and Burr ('30) and Evans et al. ('34) was not observed in our animals during 20 weeks on low-fat diets. Pseudo pregnancies appeared in two of our deficient animals. This defect was not corrected by ethyl linolate. Resorptions as reported by Maeder ('37) were not observed in any of our animals.

A comparison of the amount of water consumed did not reveal striking differences. During the twentieth week females weighing 190 to 200 gm. which received the rice-extract diet with and without fat consumed 27 and 28 cc. of water daily; those fed the yeast diet consumed 26 and 30 cc., respectively; and stock rats weighing 221 gm. consumed 30 cc. However, 28 weeks later males on the yeast diet consumed 47 cc. of water instead of 36 in spite of a drop in weight from 204 to 176 gm.

The scaliness of the hind paws and tail increased with the continued feeding of the yeast diet. At 48 weeks the hind paws were extremely scaly and somewhat erythematous; the tail was very scaly and ringed; the ears were thickened, and the testes had atrophied. Supplements of pyridoxine, pantothenic acid, or rice bran concentrate did not alleviate the symptoms. The animals became scrawny and listless and most of them died. At autopsy, kidney stones were found in 20% of them. Yet at 62 weeks four of the original fourteen male rats were still alive. Two of these had hematuria. Rice bran concentrate did not correct this but the hematuria did disappear after the administration of cottonseed oil.

Fat analyses

Fat analyses were made on the females and on all young which had reached a weight of 40 gm. The animals were killed with ether and analyzed according to the procedure described by Quackenbush and Steenbock (in press). The liver and carcass were analyzed separately (table 4).

The animals on the yeast diet contained less fat than those on the rice-extract diet, but the iodine number was higher.

However, the trend of results after feeding individual fatty acids was the same as on the rice-extract diet. The group receiving ethyl linolenate did not differ in fat content nor in iodine value from those which had received the essential fatty acids. Although unsaturated fat was evidently synthesized by the animals which did not receive dietary fat, and also by those which received ethyl linolenate, it was apparently not of an essential character.

TABLE 4

Analyses of body fats of adult female rats.

GROUP	SUPPLEMENT	RICE-EXTRACT DIET			YEAST DIET		
		Mean body weight	Total fat	Iodine number	Mean body weight	Total fat	Iodine number
		gm.	%		*gm.*	%	
1	No fat	202	11.0	67	186	...	..
2	Cottonseed oil	213	10.8	69	169	8.9	74
3	Ethyl linolate	196	10.7	66	201	7.6	75
4	Ethyl linolenate	192	10.3	70	196	8.3	72
5	Ethyl arachidonate	204	11.4	65	185	8.1	70
7	Ethyl linolate from 2nd week	208	10.4	68	...	...	..

The analyses of the body fats and liver fats of the young produced on the two diets likewise revealed no significant differences. The body fats ranged from 4.8 to 7.1% with iodine numbers from 60 to 78, and liver fats from 1.3 to 3.9% with iodine numbers from 77 to 112. The higher iodine numbers were found in the groups of the lower fat content.

DISCUSSION

The rice-extract diet apparently was superior to the yeast diet because it gave the better growth after the addition of essential fatty acids. This is not inconsistent with the observation of Schneider et al. ('40). However, in view of the results obtained in our long-term experiments, the interpretation of Schneider et al. ('40), that rice bran concentrate can replace the essential fatty acids in all their functions, is not sub-

stantiated. Rice bran concentrate can prevent acrodynia but it cannot prevent the scaliness of the feet and tail.

On the basal diets, failure of reproduction always ensued. After a prolonged gestation period, parturition was accompanied by excessive vaginal bleeding and loss in weight. These results are in agreement with the observations of Maeder ('37) that hemorrhages in the placenta and uterine wall are the cause of fetal death in fat deficiency.

The low percentage and the poor nutritive state of the young weaned in the experiments of others with linoleic supplements were probably due to the low levels fed. Evans et al. ('34) supplemented a yeast diet with 40 mg. of linoleic acid in the form of a concentrate from corn oil. Although this dose was increased to 120 mg. after parturition, only half of the young survived, and those weighed only 28 gm. at weaning. When 25% of lard was included in the diet over 90% survived, and the weight at weaning was 39 gm. Mackenzie, Mackenzie and McCollum ('39) fed 25 mg. of methyl linolate with a diet containing a water-soluble extract of yeast and carried their female rats through three gestation periods. No young were weaned until the second and third gestations and the young of the heaviest litter averaged 31.0 gm. at 21 days. It is possible that the level of methyl linolate was inadequate for the first gestation, but due to a gradual and tenacious storage (Sinclair, '35) sufficient amounts of the essential fatty acids accumulated for the later gestations. In view of these results it is recommended that in reproduction and lactation studies rats should receive daily at least 100 mg. of the essential fatty acids or their equivalent in the form of a natural oil. Turpeinen ('38) has already recommended the use of 100 mg. for maximal growth.

Maeder ('37) has stated that "failure of reproduction in humans due to lack of adequate fat in the diet is considered possible." It is not excluded that reported beneficial effects of wheat germ oil in human pregnancy may have been due in part to meeting the essential fatty acid requirements.

The results of the reproduction studies parallel those obtained when dermal lesions were used as criteria. When assayed with acrodynic rats as described by Quackenbush et al. ('39), 10 mg. daily of either linoleic or arachidonic acid cured the dermal symptoms. As these animals weighed 50 gm. and our females weighed 200 gm., the requirement per kilogram of body weight is about twice as high for reproduction as for the cure of dermal lesions. Linolenic acid was ineffective in both instances. Burr, Brown, Kass and Lundberg ('40) have reported that linolenic acid gave good growth responses but had little effect on the skin. Arachidonic and linoleic acids were similar to each other in their effects. Our data on reproduction as well as on acrodynia have revealed no significant difference of physiological activity between linoleic and arachidonic acids; however, they corroborate abundantly the practical inability of linolenic acid to correct the symptoms of fat deficiency (Hume et al., '38).

CONCLUSIONS

1. On a low-fat diet furnishing only 3.0 mg. of unsaturated lipid or a calculated maximum of 0.8 mg. of linoleic acid per rat per day, rats were raised to maturity and bred. After a prolonged gestation period and severe hemorrhage in parturition, the young were born dead or died soon after birth. A scaly condition of the hind paws and tail was observed after about 10 weeks on the diet.

2. Ethyl linolate and ethyl arachidonate prevented or cured the dermal symptoms completely and produced normal young which were weaned at the age of 3 weeks with an average body weight of 40 gm. The requirement for these acids appears to be higher than previously estimated.

3. Ethyl linolenate did not make possible the production of normal young; neither did it cure the dermal symptoms.

4. Fat analyses revealed a remarkable constancy in both the percentage of total fat and the iodine values of the fat, irrespective of the dietary supplements.

LITERATURE CITED

BURR, G. O., AND M. M. BURR 1930 On the nature and role of the fatty acids essential in nutrition. J. Biol. Chem., vol. 86, p. 587.

BURR, G. O., M. M. BURR AND E. S. MILLER 1931 On the fatty acids essential in nutrition. J. Biol. Chem., vol. 97, p. 1.

BURR, G. O., J. B. BROWN, J. P. KASS AND W. O. LUNDBERG 1940 Comparative curative values of unsaturated fatty acids in fat deficiency. Proc. Soc. Exp. Biol. Med., vol. 44, p. 242.

EVANS, H. M., S. LEPKOVSKY AND E. A. MURPHY 1934 Vital need of the body for certain unsaturated fatty acids. J. Biol. Chem., vol. 106, p. 431.

HUME, E. M., L. C. A. NUNN, I. SMEDLEY-MACLEAN AND H. H. SMITH 1940 Fat-deficiency disease of rats. Biochem. J., vol. 34, p. 879.

———— 1938 Studies of the essential unsaturated fatty acids in their relation to the fat-deficiency disease of rats. Biochem. J., vol. 32, p. 2162.

MACKENZIE, C. G., J. B. MACKENZIE AND E. V. MCCOLLUM 1939 Growth and reproduction on a low-fat diet. Biochem. J., vol. 33, p. 935.

MAEDER, E. C. 1937 The effect of fat in simplified diets on the reproductive organs of the female albino rat during gestation. Anatomical Record, vol. 70, p. 73.

NEWMAN, M. S., AND R. J. ANDERSON 1933 The chemistry of the lipids of yeast. 1. The composition of the acetone-soluble fat. J. Biol. Chem., vol. 102, p. 219.

QUACKENBUSH, F. W., B. R. PLATZ, AND H. STEENBOCK 1939 Rat acrodynia and the essential fatty acids. J. Nutrition, vol. 17, p. 115.

QUACKENBUSH, F. W., AND H. STEENBOCK Body fats in rat acrodynia. (J. Nutrition, in press.)

ROLLETT, A. 1909 Zur Kenntnis der Linolsaure. Zeitschrift f. Physiol. Chemie., vol. 62, p. 411.

SCHNEIDER, H., AND H. STEENBOCK 1939 A low phosphorus diet and the response of rats to vitamin D_2. J. Biol. Chem., vol. 128, p. 159.

SCHNEIDER, H., H. STEENBOCK AND B. R. PLATZ 1940 Essential fatty acids, vitamin B_6, and other factors in the cure of rat acrodynia. J. Biol. Chem., vol. 132, p. 539.

SHINOWARA, G. Y., AND J. B. BROWN 1940 Study on the chemistry of fatty acids. VI. The application of crystallization methods to the isolation of arachidonic acid, with a comparison of the properties of this acid prepared by crystallization and by debromination. Observations on the structure of arachidonic acid. J. Biol. Chem., vol. 134, p. 331.

SINCLAIR, R. G. 1935 The metabolism of the phospholipids. VII. Further evidence of the selection and retention of unsaturated fatty acids by phospholipids of animal tissues. J. Biol. Chem., vol. 111, p. 275.

SMEDLEY-MACLEAN, I. 1922 The conditions influencing the formation of fat by the yeast cell. Biochem. J., vol. 16, p. 370.

STEENBOCK, H. 1923 A satisfactory ration for stock rats. Science, vol. 58, p. 449.

TURPEINEN, O. 1938 Further studies on the unsaturated fatty acids essential in nutrition. J. Nutrition, vol. 15, p. 351.

LINOLEIC ACID, PYRIDOXINE AND PANTOTHENIC ACID IN RAT DERMATITIS [1, 2]

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Studies in numerous laboratories have shown that rat dermatitis in its various forms is etiologically related to a number of deficiencies. The work of Goldberger and Lillie ('26) emphasized the importance of a water-soluble substance in preventing or curing dermal lesions. The findings of Burr and Burr ('29) focused attention on fats and constituent fatty acids. Richardson and Hogan ('36), Birch and György ('36) and Salmon ('38) demonstrated that water-soluble and lipid factors were both involved in the prevention and cure of the severe form of dermatitis now frequently termed "rat acrodynia." Tests with isolated compounds have shown that linoleic acid (Quackenbush et al. '39), pyridoxine (György, '38; Lepkovsky, '38), and pantothenic acid (György et al., '39) are factors involved in the production and cure of the disease. These findings have been substantiated by other investigators. However, the effects of the individual factors have not been clearly demonstrated since the basal diets usually contained considerable quantities of either unsaturated fat or crude concentrates of the water-soluble factors. The present report deals with the interrelationships observed when the afore-

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mentioned supplements were given as pure compounds superimposed on a basal diet lacking all of them.

EXPERIMENTAL

All the experiments were performed with rats. In preparation for experiment, litters were raised to weaning age on a diet consisting chiefly of potato meal (Quackenbush et al., '39). They were weaned at a weight of 38 to 42 gm. and placed on the basal diet (diet 5) or, in some of the prophylactic studies, on a modification of this diet. Diet 5 had a percentage composition of glucose [3] 78, casein [4] (alcohol-extracted) 18, and salts [5] 4. In addition each rat was given a daily dose of 10 μg. of thiamine and 20 μg. of riboflavin [6] dissolved in 1 drop of 0.02 N acetic acid, and 10 μg. of carotene and 5 μg. of calciferol dissolved in 1 drop of liquid hydrogenated coconut oil (I. V. = 0.1). The casein carried a small amount of unsaturated lipid (0.11%, I. V. = 40.0) extractable after acid hydrolysis. In all, the basal diet contained no more than 0.003% of unsaturated lipid calculated as linoleic acid.

The test supplements were administered by dropper. Pyridoxine hydrochloride [6] and calcium d-pantothenate [6] were fed as synthetic compounds in aqueous solution. The ethyl linolate was prepared from corn oil via the tetrabromide (m. p. 115.0°). The freshly distilled ester (I. V., Wijs, 164.0) was sealed in glass ampules under high vacuum. After an ampule was opened for use its contents were kept under nitrogen in a refrigerator between feedings. A fresh supply was provided from a new ampule every 3 or 4 days.

In evaluating the effects of the various supplements, attention was given not only to the acrodynia in its various stages but to all phases of erythematous dermatitis as well as

[3] Cerelose.

[4] Labor for the purification of the casein was furnished by the Works Progress Administration.

[5] Wesson.

[6] The pyridoxine, pantothenic acid and riboflavin were generously supplied by Merck and Company.

to the dry scaliness which often persisted after all evidence of inflammation had disappeared. The term "acrodynia" was applied to both the acute form (Quackenbush et al., '39) characteristic of animals on our basal diet, and the chronic form of "rat acrodynia" (Birch et al., '35) which is produced on diets partially deficient in the essential fatty acids. The chronic form developed less rapidly, involved larger areas of the peripherae, and lacked the topographical uniformity of distribution as well as the severity of the acute form. The term "dry scaliness' was applied to cases of exfoliation in which erythema and exudation were not apparent. This condition resembled that which develops on a low-fat diet containing yeast or rice bran concentrate (Burr and Burr, '29; Quackenbush et al., '42), and which is commonly attributed to a fat deficiency.

To facilitate comparison of the degree of dermal involvement, initially and during the progress of the tests, the extent and severity of the lesions were expressed as a dermal index. This index was a numerical expression of the stage of development of the lesions of the lips, eyelids, fore-paws, hind-paws, ears and tail. It was obtained by recording the stage of development at each of these foci in accordance with our previous method (Quackenbush et al., '39) and adding the recorded numbers. The sum, or dermal index, had a theoretical maximum of 23. In practice the highest observed before death was 16.

Therapeutic relations

The weanling rats placed on the basal diet developed a severe acrodynia in 4 to 5 weeks. The test supplements were administered when the animals had a dermal index of 5 to 6, at which time the body weight usually ranged from 50 to 60 gm. Daily supplementation was continued for a period of 3 or more weeks. A cure of acrodynia was assumed to have been effected when the lips, eyes and ears were normal in appearance and the paws and tail free from all symptoms except for a dry scaliness, i.e., a dermal index of 1 or 2.

The therapeutic results obtained with both single and multiple supplements are summarized in table 1.

When fed alone, pantothenic acid produced no improvement. Pyridoxine tended to arrest the development of acute symptoms; it extended the period of survival and thereby permitted the chronic form to develop. An early improvement in the condition of the lips and fore-paws was followed by an intensification of dermal lesions of both the hind- and fore-paws. Lesions also appeared on the ears and tail.

TABLE 1

Curative effects of single and multiple supplements.

DAILY SUPPLEMENT				NO. OF RATS	MEAN 3-WEEK GAIN [1]	MEAN DERMAL INDEX AT		
Group	Ethyl linolate	Pyridoxine	Pantothenic acid			Start	3-weeks [1]	6 weeks [1]
	mg.	*µg.*	*µg.*		*gm.*			
1	..	..	..	10	—7	5	11	....
2	..	2	..	4	4	5	6	8 (4)
3	..	4	..	4	5	5	4	5 (3)
4	..	6	..	4	13	6	7	5 (3)
5	..	10	..	4	3	5	5	6 (4)
6	..	20	..	68	6	6	7	6 (9)
7	..	..	50	11	—4	5	13	....
8	5	..	..	11	—2	6	6	....
9	10	..	..	19	0	6	1	1
10	160	..	..	5	2	5	2	....
11	..	20	50	16	26	5	4	5 (4)
12	10	20	..	6	10	6	2	....
13	10	..	50	3	11	6	3	1 (3)
14	10	10	50	3	59	7	2	2 (3)
15	6	6	50	3	66	5	2	2 (3)
16	5	5	50	6	29	5	2	....
17	5	5	..	5	5	5	2	....
18	4	4	50	4	51	6	2	2 (4)
19	2	2	50	4	30	5	3	3 (4)
Rice bran concentrate [2]								
20	200	..	..	13	33	5	2	....

[1] The figures include only those animals which survived the full period. After the third week some animals were taken for other experiments, especially from the larger groups; hence in the data for 6 weeks effective totals are given in parentheses.

[2] Vitab.

Linoleic acid when fed as the ethyl ester at a subcurative level, viz., 5 mg. daily, produced an effect similar to that obtained with pyridoxine. When fed at higher levels, viz., 10 mg. or more, the acrodynia was cured completely. The final dermal index of 1 or 2 was due to a dry scaliness of the tail and, in some cases, of the hind-paws.

Pyridoxine together with pantothenic acid produced a greater improvement than was obtained with pyridoxine alone, but less than linoleic ester or a commercially available rice bran concentrate [7]. The dermal symptoms commonly persistent after this supplement were a narrow, denuded, slightly swollen erythematous margin surrounding the lips; a similar denuded, slightly scaly margin about the eyelids, thus sometimes giving a "spectacled" appearance; a dry scaliness on the dorsal side of the paws, with a slight erythema between digits; scaliness of the ears which were shriveled in appearance and shiny in exfoliated areas; and a scaliness of the tail, with ringlets, especially near the tip. Five drops of cottonseed oil daily cured these dermal symptoms except for a persistent dry scaliness of the hind paws and tail.

Ethyl linolate together with pyridoxine and pantothenic acid (group 14) cured the acrodynia completely, but a dry scaliness persisted just as it did when cottonseed oil was given with these water-soluble supplements.

When linoleic ester was fed in amounts insufficient to reduce the dermal index, the addition of pyridoxine brought about a definite improvement (groups 15 to 19). This result was not enhanced by the addition of pantothenic acid. These data leave no doubt concerning the existence of a supplementary effect between linoleic acid and pyridoxine.

From the above findings emerges the outstanding fact that for the cure of acrodynia under our experimental conditions linoleic acid was the primary indispensable curative factor. When present in sufficient amounts it cured the acrodynia without the addition of pyridoxine; when present in subcurative amounts its effectiveness was increased by the addition of pyridoxine.

[7] Vitab.

Prophylactic relations

To determine the effect of various factors on the prevention of dermal lesions, five groups of rats were given supplements to the basal diet prior to the development of dermal abnormalities (table 2). The supplements were given from the time of weaning to all animals except those in group 23 which received the supplements after the third week.

Twenty or 40 μg. of pyridoxine daily produced a marked retardation in the development of lesions. None of the animals developed a severe acrodynia within 5 weeks, and many had

TABLE 2

Effectiveness of dietary factors in preventing dermal lesions.

GROUP	DIET V ALTERATIONS				NO. OF RATS	MEAN DERMAL INDEX AT		
	pyridoxine	pantothenic acid	corn oil	casein		5th week	12th week	16th week
	μg.	*μg.*	%	%				
21	20	..	..	..	27	2	..	..
22	..	50	..	..	10	5	..	..
23	20	50	..	..	4	1	5	..
24	..	50	3	..	10	0	1	2
25	..	50	3	9	10	0	1	1

not attained a dermal index of 6 by the ninth week. During this period the mortality rate was high and a considerable number of rats succumbed without developing acrodynia. In general, the time required to produce severe lesions was longer than that required to obtain comparable lesions in relapsed animals in the curative series. Growth during the first 5 weeks was only slightly better than that of animals on the basal diet.

In marked contrast to pyridoxine, pantothenic acid did not retard the development of acrodynia. However, the two together retarded the development of symptoms more effectively than pyridoxine alone. The dermal symptoms were indistinguishable from those described for animals which received therapeutically pyridoxine together with pantothenic acid.

On diets lacking pyridoxine but supplying linoleic acid (corn oil) and pantothenic acid, animals did not develop acrodynia but grew poorly. At the twelfth week the mean body weights of rats in groups 24 and 25 were 88 and 72 gm., respectively; thereafter no appreciable growth resulted in either group. At the sixteenth week three animals in group 24 and one in group 25 showed a dry scaliness of the ears, hind-paws and tail. Thereafter, the mortality rate was high.

DISCUSSION

Essential fatty acids hold a dominant position in acrodynia since when fed alone they can prevent or cure it. However, in the maintenance of a normal dermal condition other factors including pyridoxine, pantothenic acid, and at least one as yet unidentified factor are operative. This is evidenced in a number of ways: Pyridoxine can delay the appearance of symptoms and in some cases produce temporary healing. It can increase the efficiency of linolate to diminish the amount required to cure acrodynia. Pyridoxine together with pantothenic acid can alleviate or prevent the development of severe dermal lesions on a diet virtually free from essential fatty acids. Rice bran concentrate, supplying still other substances, can limit the dermal lesions to a mild dry scaliness.

Our preliminary report (Quackenbush et al., '41) concerning these experiments appeared simultaneously with reports from other laboratories (Salmon, '41; Richardson and Hogan, '41). Their conclusions, while for the most part in accord with ours, differ with respect to the curative effect of essential fatty acids when both pyridoxine and pantothenic acid are lacking. Richardson, Hogan and Itschner ('41) recently published their results in detail. They found that the dermatitis produced in rats fed diets virtually free from fat was not cured by 5 mg. of either linoleic acid or methyl arachidonate daily, and that variable results were obtained with higher levels of these supplements. The ineffectiveness of 5 mg. of either compound is in agreement with our results. Likewise the unreliability of free linoleic acid as a curative agent, even

when given in larger doses, is in agreement with our earlier observations (Quackenbush et al., '39). Their results with methyl arachidonate are of questionable significance since only two rats were given this supplement at levels higher than 5 mg. One of these was fed 10 mg. together with pyridoxine; it gave no response to either supplement. The other, which was fed 50 mg., was stated to have healed temporarily.

The results obtained by Richardson et al. ('41) with pyridoxine together with pantothenic acid also agree essentially with our data in respect to the persistence of the dermal symptoms. They reported that rats which were cured when given either linoleic acid or methyl arachidonate later developed dermatitis. We also observed mild erythematous lesions in one rat (group 25) and have previously observed relapses in four out of thirty-six rats from 12 to 15 weeks after they had been cured with oil supplements. However, these four rats failed to respond when given pyridoxine together with pantothenic acid, and the possibility is not excluded that after a multiple deficiency of such long standing, the animals were in a physiological state resistant to therapeutic measures.

The curative action of certain natural fats and purified esters of linoleic and arachidonic acids has been observed in a large number of animals. Our experience has shown that greater precautions must be taken to obtain consistent results with purified esters than with natural oils. The simple esters, unlike glycerides, have a tendency to cause loss of hair when spilled on the cheeks and chin; hence, care must be taken to place the supplement directly into the mouth if this is to be avoided. The purified esters contain no antioxidant and consequently oxidize rapidly. Esters having an appreciable peroxide number are not well received by the rat and are not as effective as the fresh material. Free fatty acids are exceedingly irritating.

Other important factors in determining the response of the rat to supplements are the stage of acrodynia and the weight of the animals at the time therapy is begun. Rats with a dermal index of 5 or 6 usually respond to potent supplements,

but if the index is allowed to advance beyond 6 the probability of response diminishes. Similarly, rats weighing 50 gm. or more show a higher percentage of response than rats of similar age weighing 35 or 40 gm. In therapeutic studies we have seldom used acrodynic animals with body weights of less than 50 gm.

The relationships of the different factors to growth have received little consideration in the present discussion. While each has been shown to have demonstrable effects, by far the greatest growth effects of single supplements were produced by the water-soluble factors.

All the factors except essential fatty acids are apparently supplied by rice bran concentrate. Since this material without a supplement of essential fatty acids can limit the dermal lesions to a dry scaliness of the tail and paws (Quackenbush et al., '42), it is more effective than pyridoxine plus pantothenic acid. This fact is taken as evidence for the existence of an additional water-soluble factor. Schneider et al. ('40) reported that an "accessory" factor present in rice bran concentrate was needed to supplement pyridoxine in curing acrodynia. It now becomes evident that their "accessory" factor was multiple in nature, one component being pantothenic acid. Whether the additional factor is a known dietary essential remains to be determined.

SUMMARY

Rat acrodynia was produced on a diet containing 0.003% of unsaturated fat. Various factors were tested for their curative action. Pantothenic acid did not even alleviate the symptoms. Pyridoxine produced temporary alleviation but did not effect a cure. Ethyl linolate cured the acrodynia. Amounts of linolate which were subcurative alone became curative when given with pyridoxine. Pantothenic acid together with pyridoxine improved the dermal condition, and linolate subsequently produced further improvement. The three compounds together cured the acrodynia but did not cure completely the scaly condition of the tail and hind-paws. The results indicate that an additional factor is involved.

In prophylactic tests neither pantothenic acid nor pyridoxine prevented the acrodynia but pyridoxine retarded the development of the dermal lesions. A lack of pyridoxine did not result in acrodynia when animals were fed both linoleic acid (corn oil) and pantothenic acid.

Sustained growth resulted only when all three supplements were fed.

LITERATURE CITED

BIRCH, T. W., AND P. GYÖRGY 1936 A study of the chemical nature of vitamin B_6 and methods for its preparation in a concentrated state. Biochem. J., vol. 30, p. 304.

BIRCH, T. W., P. GYÖRGY AND L. J. HARRIS 1935 The vitamin B complex. Differentiation of the antiblack-tongue and the "P.-P." factors from lactoflavin and vitamin B_6 (so-called "rat-pellagra" factor). Parts I–VI. Biochem. J., vol. 29, p. 2847.

BURR, G. O., AND M. M. BURR 1929 A new deficiency disease produced by the rigid exclusion of fat from the diet. J. Biol. Chem., vol. 82, p. 345.

GOLDBERGER, J., AND R. D. LILLIE 1926 A note on an experimental pellagra-like condition in the albino rat. Pub. Health Rep., U. S. P. H. S., vol. 41, p. 1025.

GYÖRGY, P. 1938 Crystalline vitamin B_6. J. Am. Chem. Soc., vol. 60, p. 983.

GYÖRGY, P., C. E. POLING AND Y. SUBBAROW 1939 Experiments on the anti-dermatitis component of the filtrate factor in rats. Proc. Soc. Exp. Biol. Med., vol. 42, p. 738.

LEPOVSKY, S. 1938 Crystalline factor 1. Science, vol. 87, p. 169.

QUACKENBUSH, F. W., F. A. KUMMEROW AND H. STEENBOCK 1941 Fat metabolism in rat acrodynia. J. Nutrition, Proc. Am. Inst. Nutrition, vol. 27, p. 11.

———— 1942 Effectiveness of linoleic, arachidonic and linolenic acids in reproduction and lactation. J. Nutrition, vol. 24, p. 213.

QUACKENBUSH, F. W., B. R. PLATZ AND H. STEENBOCK 1939 Rat acrodynia and the essential fatty acids. J. Nutrition, vol. 17, p. 115.

RICHARDSON, L. R., AND A. G. HOGAN 1936 Skin lesions of the rat associated with the vitamin B complex. Mo. Agr. Exp. Sta. Res. Bul., vol. 241.

———— 1941 Factors related to rat dermatitis. J. Biol. Chem., vol. 140, p. cvi.

RICHARDSON, L. R., A. G. HOGAN AND K. F. ITSCHNER 1941 Vitamin B_6, pantothenic acid, and unsaturated fatty acids as they affect dermatitis in rats. Research Bulletin, University of Missouri, College of Agriculture, Agricultural Experiment Station, 333. September, pp. 1–12.

SALMON, W. D. 1938 The effect of certain oils in alleviating localized erythematous dermatitis (acrodynia or vitamin B_6 deficiency) in rats. J. Biol. Chem., vol. 123, p. civ.

———— 1941 The relation of pantothenic acid, pyridoxine, and linoleic acid to the cure of rat acrodynia. J. Biol. Chem., vol. 140, p. cix.

SCHNEIDER, H., H. STEENBOCK AND B. R. PLATZ 1940 Essential fatty acids, vitamin B_6, and other factors in the cure of rat acrodynia. J. Biol. Chem., vol. 132, p. 539.

NICOTINIC ACID IN FOODS

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Although the clinical use of nicotinic acid has received wide publicity and its physiological action is frequently discussed, yet it is only comparatively recently that the practical problem of its quantitative occurrence has been attacked. One difficulty has been that the present methods of assay are far from being perfect, and frequently give results which are not always capable of substantiation.

It is, therefore, not surprising to find a lack of acceptable average nicotinic acid values such as might be found in tables of food composition. Study of the literature disclosed that, as mentioned above, different methods of assay gave somewhat divergent results. Moreover, marked variations in nicotinic acid values for the same type of item could occur naturally; thus, for example, the previous diet of an animal could play a major role in determining the nicotinic acid content of the animal's tissues. It was, however, necessary to obtain such estimated average values for common foods in so far as our present knowledge would permit. It is in the belief that a résumé of the literature on this subject may prove useful to other workers, that the following table is presented.

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TABLE 1

Nicotinic acid content — mg./100 gm. fresh weight.

FOOD	MICROBIOLOGICAL		BIOLOGICAL		CHEMICAL METHOD	
	Value	Ref.	Value	Ref.	Value	References
***MEATS* — Fresh**						
Beef						
Brain (4.9)	*15.0	31	7.5	29	4.9, 2.4, 3.0, 6.0	29, 9, 15, 17
Heart (7.6)	*39.0	31	4.9	29	4.9, 5.92, 11.4, 7.6	9, 4, 17, 29
Kidney (7.34)	*32.0	29	16.2	29	7.34, 4.4, 5.8	29, 9, 4
	*26.0	31			10.2, 5.8	17, 19
Liver (17.9)	*47.0	29	26.5	29	9.2, 17.0, 15.5–20	2, 15, 16
	*40.0	31			12.2, 17.7, 17.9	4, 17, 29
					17.7, 7.7 & 25.0	18, 19
					8.6, 20.2	9, 28
Lung (6.2)	*11.0	31	8.3	29	6.2, 6.6	29, 17
Muscle (6.39)	*22.0	31	3.8	29	4.3, 4.9, 4.9, .02	15, 16, 4, 12
			10.2	29	4.9, 4.8, 6.39, 4.9	17, 7, 29, 18
					3.5, 4.0, 2.5, 4.8	19, 9, 2, 28
Spleen (3.2)	*20.0	29	9.2	29	3.2, 6.2, 4.7	9, 17, 28
	* 8.9	31				
Tongue (7.1)			12.8	29	6.12, 7.1	29, 17
Various cuts						
Pancreas (5.84)	*15.7	29	3.2	29	5.84	29
Round					7.86	29
Fowl						
Chicken (11.2)					10.0–13, 11.2–13.5	18, 17
					4.2	9
Dark					6.14	29
Light (7.3)	*29.0	29			7.3	29
Liver (8.0)					8.0–9.6, 6.2	17, 9
Heart (7.4)					7.4, 2.9	17, 9
Grouse — fresh					6.55	18
Liver					13.3	17
Heart					6.9	17
Rabbit						
Brain					1.2	23
Heart					3.4	23
Liver (14.3)					7.85, 14.3	23, 29
Lung					0.9	23
Muscle (6.5)					11.0, 6.5	29, 23
Mutton and *Lamb*						
Muscle (4.5)					4.5, 4.1, 2.8	18, 19, 9
Adrenals					13.5	15
Brain (3.2)					2.6, 3.2, 4.7	19, 9, 17
Heart (6.0)					8.7, 4.4, 6.0, 5.5	19, 9, 15
						17
Kidney (6.0)					6.0, 7.5, 5.4	9, 15, 17
Liver (17.6)	*42.0	29	42.5	29	10.1, 20.0	9, 15
					12.5, 17.6, 12.5	17, 29, 18
					15.5 & 25.2, 10.1	19, 8
Lung					3.7	17
Pancreas					4.0	15
Spleen					3.8	9
Various cuts						
Leg					8.48	29
Pork						
Brain (6.4)	*19.0	31			6.4, 10.0	9, 17
Heart (7.3)	*24.5	31	8.0	29	7.3, 5.34, 7.8	9, 4, 17
Kidney (9.8)	*45.5	29	15.5	29	9.8, 7.2, 6.82, 7.6	29, 9, 4, 17
Liver (22.8)	*53.4	24			28.2, 15.7	28, 17
	*54	29	27.0	29	22.8, 15.7, 16.0	29, 18, 9
	*39	31			11.8	4
Lung	*14	31			2.5	17

* All figures with asterisk on dry basis — others fresh.

TABLE 1 — (*Continued*)

FOOD	MICROBIOLOGICAL		BIOLOGICAL		CHEMICAL METHOD	
	Value	Ref.	Value	Ref.	Value	References
Pork						
Muscle	*18.0	31			0.02, 3.1, 5.3	12, 17, 28
					3.13, 2.6, 6.2, 3.3	18, 19, 9, 16
					4.73	4
Pancreas					5.0	4
Spleen (4.5)	*12.0	31			5.3, 4.5	9, 17
Stomach					3.0	17
Thymus					3.25	4
Tongue					5.3	17
Various cuts						
Bacon					4.4	19
Ham (8.8)			8.8	29	7.14	29
Loin (8.8)	*23.5	29	8.8	29	6.4	29
Smoked ham (8.2)	14.5	29	8.2	29	5.7, 8.3	29, 28
Tenderized ham (8.3)	*16.0	29	8.3	29	5.8	29
Veal						
Heart					10.6	29
Liver (17.6)	*50	24	22.5	29	17.6	29
	*41.0	29				
Various cuts						
Hindquarters			6.5–17.0	29	8.4	29
Fish						
Cod — Flesh (2.3)					1.7, 3.0, 1.7, 1.95	18, 15, 16, 4
					2.3	29
Liver					1.6	18
Roe (1.52)					1.4, 1.52	16, 4
Crab (2.8)					2.6, 2.82, 2.8	19, 18, 17
Cuttle fish					0.9	19
Flounder					3.84	18
Halibut					6.08	18
Herring (3.5)					3.5–4.0, 4.0, 2.9	18, 15, 16
Milt (2.36)					2.36, 2.1	18, 15
Roe (2.36)					2.36, 2.1	18, 15
Ling					2.7	17
Mackerel (5.5)					5.5, 7.2	18, 17
Mullet					2.9	7
Oyster					1.3	19
Salmon (6.0)					6.0, 8.4, 7.35	18, 15, 29
COOKED OR CANNED MEATS AND FISH						
Beef						
Heart (7.2)					3.3 & 7.2	29
Kidney, stewed (5.23)					5.23	29
Liver, fried (15.8)	*38.0	29	29.4	29	15.8	29
Round, fried (9.05)					9.05, 4.2	29, 19
Roasted (4.28)			10.2	29	4.28	29
Boiled 20 min. (2.4)					2.4	19
Spleen, stewed	*15.8	29			6.3	29
Fowl						
Duck, roasted					2.8	19
Chicken, roasted					7.5	19
Canned					7.4	17
Liver, boiled 20 min. (11.2)					7.2 & 11.2	19
Goose, roasted					2.8	19
Heart, stewed					1.5	19
Liver, boiled 20 min.					3.6	19
Pork						
Brain, boiled 15 min.					2.2	19
Ham, fried	*14.0	29			3.7	29
Boiled (6.3)	*13.0	29	5.2	29	6.3	29

* All figures with asterisk on dry basis — others fresh.

TABLE 1 — (*Continued*)

FOOD	MICROBIOLOGICAL		BIOLOGICAL		CHEMICAL METHOD	
	Value	Ref.	Value	Ref.	Value	References
Pork						
Ham, smoked						
Fried	*13.9	24			14.3, 1.3	29, 19
Kidney, fried					4.0	19
Liver, fried					5.1 & 11.2	19
Loin, broil					4.45	29
Loin, fried					5.57	29
Sausage, fried					1.8	19
Mutton						
Boiled, 20 min.					3.9	19
Veal						
Steak, fried					8.38	29
Fish						
Brisling sardines in olive oil					3.5	17
Clam, canned	1.08	32				
Cod, canned					1.7	16
Cod roe, preserved (1.4)					0.9, 1.4	17, 16
Herring sardines (2.9)					3.35, 2.9	18, 16
Kippers, preserved					3.4	17
Oysters, canned	0.66	32				
Salmon, canned (6.0)					6.0, 2.7	16, 19
Shrimp	0.78	32				
EGGS						
Hens, white (0.076)	0.076	32	<2.5	30	<0.05, 1.02, 0.3	15, 7, 19
Yolk (0.035)	0.035	32	<4	30	tr, 1.0, 3.28, tr	18, 15, 7, 17
Duck, boiled					0.2 & 0.3	19
MILK AND PRODUCTS						
Cheese, cheddar					0.2	19
Condensed milk (0.18)	0.18	32			0.4	19
Fresh milk (0.1)	0.084	24			0.1, 0.05 0.5)	19, 17
	0.08	32			0.054, 0.1, 0.45, (0.1–	18, 27, 2, 15
					0.76, 0.82	7, 28
Klim (whole milk) powdered					1.1	27
Skim milk					0.06–.09	22
Skim milk, powdered (0.88)	0.88	32	4.3–6.2	30	1.0, 1.4 to 2.8	27, 22
			5–15	20	2.5, 1.2	15, 19
CEREALS AND PRODUCTS						
Bread						
White (0.66)	0.66–0.95	32			<0.5, 2.16, 0.2, 4.7	15, 6, 19, 7
Brown (1.80)	1.80	32			1.2, 0.8	15, 19
Whole meal (2.88)	2.88	32			0.7	19
Cereals						
Wheat (4.0)	4.0–4.9	32			3.1, 3.8, 4.7–5.3, 3.9	8, 27, 1, 7
Oats (1.1)	1.1	32			2.6–1.0	19, 1
Corn, yellow (dry whole)						
(1.56)	2.1	24			1.1, 1.3	7, 18
	1.56–2.6	32			0.2–0.8, 1.4–2.2	8, 19
					1.5, 1.4, 1.3	27, 1, 16
Corn, white (1.0)					1.3–1.6, 1.3, 1.0	27, 1, 7
					1.6, 0.7–1.5	1, 25
					4.4, 2.8, 5.6	8, 7, 9
Wheat flour, white (0.9)	5.2	24			.81	6
	0.9–1.1	32			1.3, 0.9–1.1, 4.4–3.8	19, 1, 7
Sorgum (*sorgum vulgare*)					2.2, 2.1	8, 25
Buckwheat	4.4	32				
Rye flour, whole (1.22)	6.3	24				
	1.22	10			1.3	16
Rye flour, white unbleached	0.71	10				
Rye flour, white bleached	0.73	10				

* All figures with asterisk on dry basis — others fresh.

TABLE 1 — (*Continued*)

FOOD	MICROBIOLOGICAL		BIOLOGICAL		CHEMICAL METHOD	
	Value	Ref.	Value	Ref.	Value	References
CEREALS AND PRODUCTS						
Rye flour, degerminated						
bleached	0.73	10				
Rice, flour					2.4	1
White, polished (0.90)	0.90	32			0.9, 1.5, 3.1	19, 27, 7
Parboiled, then polished (3.0)					2.6, 3.0, 3.8	19, 27, 1
Rice, unpolished (6.0)					6.0–6.4	19
Milled (1.6)					1.7, 2.1–3.2, 1.6	26, 8, 1
Millet (0.8)					0.8, trace	27, 8
Rye (1.1)	0.9–1.3	32				
Barley (4.7)	4.7	32			2.5–3.0	1
Flour	5.7	24				
Oatmeal (0.6)	1.0–1.5	24			0.6	19
Pearl Barley (2.7)	2.7	32			3.5	19
Corn meal, yellow (0.6)	0.6–1.0	24				
	0.6	24			0.6–0.8, 1.0	15, 7
	1.0	32				
White (0.9)	1.76	32			0.9	1
Flour					0.3	1
Spaghetti (2.1)	2.1	32			3.28	7
Tapioca — boiled 20 min.					0.3	19
BEANS AND OTHERS, DRIED						
Kidney bean (2.8) (*phasoleus vulgaris*)	2.8	32			2.8	7
Lima beans	1.83	32				
Broad bean					2.1	7
Soy bean (4.32*					1.2, 4.32, 4.25	19, 3, 25
Mung bean (*phasoleus aureus*)					2.3	8
Lentils					3.1	7
Peas (1.8)					2.0, 1.0, 1.8	7, 1, 1
chick (*cicer arientinum*)					3.89	7
Peanuts, raw					5.9	19
Peanut butter, 6% fat					20–23	2
Norm. fat	18.6	32				
Meal extracted (17.2)	17.2	24	13	30	16.7, 22	25, 2
Coconut, raw					0.4	19
Nuts, (unspecified type)					1.29	7
Almonds					1.82	7
Chestnuts					1.17	7
VEGETABLES — LEAFY, GREEN AND YELLOW						
Bamboo shoots					0.2	19
Beans, green (0.64)	0.64	32			0.1, 0.3	19, 19
Broccoli, leaves					1.44	7
Stem (lower)					0.97	7
Cabbage (0.29)	0.29	32			0.4, 0.3	19, 15
Carrots (1.47)	1.47	32			<0.5	15
Endive					.72	7
Kohlrabi	0.27	32				
Lettuce					0.5	19
Lima beans, green (0.29)	0.29	32			0.8	19
Mangels, leaves (0.3)					0.3	17
Stems (0.13)					0.13	17
Peas, green					0.7	19
Peppers, green (0.2)					0.2, .76	19, 7
Pumpkin (0.7)					0.7	19
Spinach (0.72)	0.72	32			1.7	15
Squash, Zucchini (*Curcubita lagenaria*)					0.96	7
VEGETABLES, LEAFY, GREEN AND YELLOW, canned						
Asparagus bean					0.5	17
Beans, wax					0.6	17
French					0.05	17
Peas					1.3, 0.3	17, 19
Spinach					0.7	17

* All figures with asterisk on dry basis — others fresh.

TABLE 1 — (*Continued*)

FOOD	MICROBIOLOGICAL		BIOLOGICAL		CHEMICAL METHOD	
	Value	Ref.	Value	Ref.	Value	References
VEGETABLES, other than leafy green or yellow, fresh						
Cucumber (0.32)	0.32	32			<0.1	19
Cauliflower	0.57	32				
Eggplant (0.6)					0.6, 0.86	19, 7
Gourd					0.1	19
Onion (0.10)	0.10	32			<0.1	19
Radish					<0.1	19
VEGETABLES, other, canned						
Beets, red (0.64)	0.64	32			0.3	17
POTATOES						
White (1.18)	1.18	32			0.4, 2.0, 1.0, 1.07	19, 15, 16, 7
Sweet (1.29)	1.29	32			<0.1	19
Yam (0.67)	0.67	32				
TOMATOES						
Fresh (0.58)	0.58	32			0.1, <0.5, .99	19, 15, 17
FRUITS, other than citrus						
Apples (0.50)	0.50	32			0.2, 0.5, 1.29	19, 15, 7
Banana	0.61	32				
Berries (no type given)					1.63	7
Cranberries	1.29	32				
Dates	2.18	32				
Figs, fresh					0.63	7
Grapes, pulp					0.84	7
Peel					0.94	7
Guava					1.1	19
Peaches	0.95	32				
Pear	0.14	32				
Plum	0.56	32				
Watermelon					<0.1	19
FRUITS, dried						
Figs					1.72	7
Raisins	0.63	32				
MISCELLANEOUS						
Yeast, bakers, fresh (11.0)					11.0, 9.9, 11.0	16, 7, 17
Yeast, dried (40)	40–50	32	50	30	29–32, 25.7, 15 & 26	2, 4, 19
Yeast, brewers, dried (40)	40–60	32	34–93	30	35.5	18
					60.6, 48.3, 41	8, 27, 2
					44, 25–45	16, 17
Yeast, brewers, fresh (10.2)					10.2, 9.1	16, 15
CONCENTRATES — etc.						
Beef extract					20.4	19
Rice bran (polishings)					30.0	27
Bran concentrates					140	17
Wheat germ (4.2)			<4	30	2.7, 4.2	15, 16
Germ extracted			<6	30		
Bran (4.2)					5.0, 5.1, 4.2	16, 7, 16

* All figures with asterisk on dry basis — others fresh.

The foods have been grouped by classes according to similar nutritive content or unique contribution to the diet. Such a classification is useful because it not only makes possible a rapid and reasonably accurate evaluation of a diet, but also does away with the tedious procedure of evaluating food items singly, even though they are of similar nutritive value. The classes of foods on this basis are as follows: meats; eggs; milk and milk products; butter; other fats; grains and grain products; legumes; sugars and syrups; leafy, green or yellow vegetables; tomatoes; citrus fruits; potatoes; vegetables other than leafy, green or yellow; fruits other than citrus; dried fruits; beverages; and miscellaneous.

The values for each food as found in the literature have been arranged in columns titled according to the method of assay. Adjacent columns termed "Reference" pertain to the source of information as indicated in the numbered bibliography. Where several values have been found in the literature, we have indicated in parentheses (adjacent to the name of the item) what is believed to be a reasonably accurate average value. Usually, the values so chosen represent those obtained by North American workers on native material. It must be emphasized, however, that these decisions will bear constant revision in the light of future findings, and that they do not in any way represent a final opinion.

LITERATURE CITED

1. Aykroyd, W. R., and M. Swaminathan 1940 The nicotinic acid content of cereals and pellagra. Ind. J. Med. Res., vol. 27, p. 667.
2. Arnold, Aaron, C. B. Schreffler and A. T. Lipsius 1941 Chemical determination of nicotinic acid. Ind. and Eng. Chem., Anal. Ed., vol. 13, p. 61.
3. Ashford, Walter R., and R. H. Clark 1939 A comparison of colorimetric methods for the quantitative determination of nicotinic acid. Trans. Royal Soc. Canada, 3rd ser. 33, Sec. 3, p. 29.
4. Bandier, Erik 1939 Quantitative estimation of nicotinic acid in biological material. Biochem. J., vol. 33, p. 1130.
5. Bandier, E., and J. Hald 1939 A colorimetric reaction for the quantitative estimation of nicotinic acid. Biochem. J., vol. 33, p. 264.

6. BINA, ALBERT F., JAMES THOMAS AND ELMER B. BROWN 1941 The determination of nicotinic acid in bread and other cereal products. Cereal Chemistry, vol. 18, p. 661.
7. DELREGNA, DI F. 1939 The nicotinic acid content of food materials. Quaderni Nutriz., vol. 6, p. 368.
8. GIRI, K. V., AND B. NAGANNA 1941 An adoption method for the estimation of the nicotinic acid content of foodstuffs. Ind. J. Med. Res., vol. 29, p. 125.
9. GIRI, K. V., AND B. NAGANNA 1941 Estimation of nicotinic acid in tissue and blood. Ind. J. Med. Res., vol. 29, p. 585.
10. IDHE, AARON J., AND HENRY A. SCHUETTE 1941 Thiamin, nicotinic acid, riboflavin, and pantothenic acid in rye and its milled products. J. Nutrition, vol. 22, p. 527.
11. KARRER, P., AND H. KELLER 1938 A colorimetric determination of nicotinic acid amide. Helv. Chim. Acta., vol. 21, p. 463.
12. KARRER, P., AND H. KELLER 1938 Quantitative determination of nicotinic acid amide in animal organs. Helv. Chim. Acta., vol. 21, p. 1170.
13. KARRER, P., AND H. KELLER 1939 Determination of nicotinic acid in animal organs. Helv. Chim. Acta., vol. 22, p. 1292.
14. KODICEK, E. 1940 Estimation of nicotinic acid in animal tissues, blood, and certain foodstuffs. I. Method. Biochem. J., vol. 34, p. 712.
15. KODICEK, E. 1940 Estimation of nicotinic acid. Biochem. J., vol. 34, p. 724.
16. KRINGSTAD, H., AND T. NAESS 1939 A colorimetric method for the determination of nicotinic acid and nicotinic acid amide in foodstuffs. Z. Physiol. Chem., vol. 260, p. 108.
17. KRINGSTAD, HANS, AND FINN THORENSEN 1940 The occurrence of the antipellagra vitamin, nicotinic acid (amide) in some foods. Nord. Med., vol. 48, p. 2248.
18. KRINGSTAD, H., AND F. THORENSEN 1940 The occurrence of the antipellagra vitamin (nicotinic acid, nicotinamide) in fish and fish products. Tids. Hermetikind., vol. 26, p. 114.
19. LEONG, P. C. 1940 Nicotinic acid content in foods. J. of the Malaya Branch of the Brit. Med. Assn., vol. 4, p. 261.
20. MCKIBBIN, J. M., HARRY WAISMAN, O. MICKELSON AND C. A. ELVEHJEM 1939 Bull. Wisc. Agric. Exp. Sta., vol. 446, p. 40.
21. MELNICK, DANIEL, AND HENRY FIELD 1940 Determination of nicotinic acid in biological materials by means of photoelectric colorimetry. J. Biol. Chem., vol. 134, p. 1.
22. NOLL, C. I., AND O. G. JENSEN 1941 The chemical determination of nicotinic acid in milk and milk derivatives. J. Biol. Chem., vol. 140, p. 755.
23. RITSERT, K. 1939 On the quantitative nicotinic acid amide content of urine, tissue, and blood. Klin. Wochenschr., vol. 18, p. 934.
24. SNELL, E. E., AND I. D. WRIGHT 1941 A microbiological method for the determination of nicotinic acid. J. Biol. Chem., vol. 139, p. 675.
25. SWAMINATHAN, M. 1938 A colorimetric method for the determination of nicotinic acid in foodstuffs. Ind. J. Med. Res., vol. 26, p. 427.

26. SWAMINATHAN, M. 1941 The effect of washing and cooking on the nicotinic acid content of raw and parboiled rice. Ind. J. Med. Res., vol. 29, p. 83.
27. SWAMINATHAN, M. 1941 Cyanogen bromide method of estimating nicotinic acid. Ind. J. Med. Res., vol. 29, p. 325.
28. WAISMAN, HARRY A., AND C. A. ELVEHJEM 1941 Chemical estimation of nicotinic acid and vitamin B_6. Ind. and Eng. Chem., Anal. Ed., vol. 13, p. 221.
29. WAISMAN, HARRY A., AND C. A. ELVEHJEM 1941 The vitamin content of meat. Burgess, Minneapolis.
30. WAISMAN, HARRY A., OLAF MICKELSEN, J. M. MCKIBBIN AND C. A. ELVEHJEM 1940 Nicotinic acid potency of food materials and certain chemical compounds. J. Nutrition, vol. 19, p. 483.
31. WILLIAMS, R. J., AND OTHERS 1941 Studies on the vitamin content of tissues. The University of Texas Publication, vol. 4137, pp. 1–87.
32. TEPLY, E. J., F. M. STRONG AND C. A. ELVEHJEM 1942 The distribution of nicotinic acid in foods. J. Nutrition, vol. 23, p. 417.
33. BACHARACH, A. L. 1941 The distribution of nicotinic acid in human and animal foods. Nutr. Abs. and Reviews, vol. 10, p. 459.

A SALT MIXTURE FOR USE WITH BASAL DIETS EITHER LOW OR HIGH IN PHOSPHORUS

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Numerous salt mixtures have been used in the past to supply the necessary inorganic elements in synthetic diets used for experimental purposes. These mixtures have varied considerably in composition but usually they were used with basal diets containing casein and yeast. Both of these materials are rather high in phosphorus and when fed at the usual levels of approximately 18% and 6%, respectively, furnish appreciable amounts of this element. Recent advances in nutrition have made possible the development of experimental diets of an increasingly high degree of purity. In diets containing neither yeast nor casein, or other phosphoprotein, there is no appreciable source of phosphorus except that added as salt.

The possibility that diets low in phosphorus will be used with increasing frequency makes it necessary to have available a salt mixture which will be satisfactory under these conditions. It is also desirable to have a single salt mixture for general laboratory use which will give good results when used with basal diets either high or low in phosphorus. The construction of such a mixture would appear possible from the work of Bethke, Kick and Wilder ('32). These authors have reported good calcification on diets in which the absolute and relative amounts of calcium and phosphorus have been varied within fairly wide limits. In our work with purified

diets low in phosphorus it was necessary to have a salt mixture which would assure an ample supply of all the inorganic elements and not only produce good growth and well-calcified bones, but also allow for reproduction and lactation. Consequently, after a study of the literature concerning the needs of the rat for the various inorganic elements we constructed a salt mixture which appeared to supply these elements in amounts as near the optimum as possible. In constructing a salt mixture the following are desirable qualifications: (a) the mixture should furnish all the inorganic elements known to be indispensable for mammals and in approximately the relative amounts demanded; (b) the minimum number of salts should be used; (c) all of the salts should be inorganic; (d) the mixture should be easily prepared; (e) the base-forming elements should be only slightly in excess of the acid-forming elements; and (f) finally the exact amount of each salt chosen should be expressed in moles or a fraction of a mole to facilitate equimolecular substitution when desired.

A few years ago Hubbell, Mendel and Wakeman ('37) reported the results of using a salt mixture (salts no. 351) which is high in calcium and low in phosphorus. This mixture, which is composed of over 50% calcium carbonate, was employed by the above authors with a basal diet containing casein and a high level (8%) of yeast. Very good results in respect to both growth and bone formation were obtained when the total salt intake was only 2% of the diet, but for ordinary feeding experiments 2.5% was recommended. Hubbell and associates also used this salt mixture with diets containing lactalbumin as the protein "to determine what type of growth and what sort of bone would be produced when the only source of phosphorus was that found in the salt mixture itself." They suggested that under these conditions the total salt intake be increased to 3%. The albumin diet, however, still contained 8% of yeast. This quantity of yeast would supply approximately 0.13% phosphorus or about half the total phosphorus in the basal casein-containing diet and nearly as much as that present in the salt mixture if the latter

were fed at 3%. The results obtained with this amount of phosphorus in the basal diet cannot be interpreted as showing what might take place if the basal diet were really devoid of phosphorus and "the only source of phosphorus was that found in the salt mixture itself."

The total phosphorus of the casein-yeast diet after the addition of 2% of the salt mixture was approximately 0.345%. In order to supply this amount, if the salt mixture was the only source of phosphorus, it would be necessary to feed over 6 gm. of the salt mixture per 100 gm. of diet, and the calcium to phosphorus ratio would be greater than 4 to 1. This salt mixture has been used by other investigators rather widely, and little attention has been given to the amount of phosphorus in the basal diet. Sure [1] ('40), for example, has used this salt mixture at a 4% level with a diet containing fibrin as a protein, and the B factors, for the most part, furnished as pure substances. Such a basal diet is very low in phosphorus.

We chose salts no. 351 to compare with our salt mixture (salts no. 12) for the following reasons: (a) with the basal diet they used, Hubbell et al. found that salts no. 351 produced very good growth and calcification; (b) salt mixture 351 has been very widely used by others; and (c) because of the high calcium to phosphorus ratio it did not seem that salts no. 351 would be a satisfactory mixture to use with a basal diet low in phosphorus. Salt mixture 12 has been compared with salts no. 351 in its ability both to produce growth and calcification of bone and to maintain normal concentrations of serum calcium and inorganic phosphorus. The salt mixtures were compared at various levels using two different basal diets — one low in phosphorus and the other moderately high in phosphorus. The composition of salt mixture 12 and the results of comparing it with salts no. 351 are given below.

EXPERIMENTAL

Salt mixture 12, the composition of which is given in table 1, is quite similar to salts no. I of Phillips and Hart ('35), but

[1] Sure ('41) has recently reported poor reproduction and lactation with salt mixture 351.

the latter does not fulfill all the qualifications previously listed. Furthermore, salt mixture 12 contains more manganese [2] and a trace of cobalt, and the phosphate is supplied as the monopotassium instead of the dipotassium salt. The use of the monobasic phosphate decreases the amount of potassium and makes the salt mixture more nearly neutral. This mixture contains neither aluminum nor fluorine. There is little evidence that animals require aluminum, and Evans and Phillips

TABLE 1

Composition of salt mixture 12.

SALT	MOLS	GRAMS	ELEMENT	AMOUNT OF ELEMENT IN 4 GM. OF SALT MIXTURE [1]
$NaCl$	5	292.5	Na	0.206
KH_2PO_4	6	816.6	Cl	0.319
$MgSO_4$	1	120.3	K	0.421
$CaCO_3$	8	800.8	P	0.334
$FeSO_4 \cdot 7H_2O$	0.2	56.6	Mg	0.043
KI	0.01	1.66	Ca	0.576
$MnSO_4 \cdot 2H_2O$	0.05	9.35	Fe	0.020
$ZnCl_2$	0.004	0.5452	I	0.00228
$CuSO_4 \cdot 5H_2O$	0.004	0.9988	Mn	0.00494
$CoCl_2 \cdot 6H_2O$	0.0002	0.0476	Zn	0.00047
			Cu	0.00046
			Co	0.00002

[1] Equivalent to amount added to 100 gm. of diet if salt mixture is fed at 4%. Calculated on a dry basis.

('39) have shown that if fluorine is necessary the amount needed is so small that they were unable to demonstrate its essential nature. If thought desirable, a small amount of fluoride could be added without materially disturbing the composition of the salt mixture with respect to the other elements. The calcium to phosphorus ratio of this mixture is 1.7 to 1. Hubbell et al. obtained maximum calcification on their casein-

[2] Daniels and Everson ('35) and Richardson and Hogan ('40) have pointed out that the demand for manganese is considerably increased during reproduction and lactation. The chick also requires comparatively large amounts of manganese, and since salt mixture 12 was first constructed and used, Waisman, Mickelsen, McKibbin and Elvehjem ('40) have modified salts no. I of Phillips and Hart to contain more manganese when using it with the chick.

yeast diet with 2% of salts no. 351. This diet contained a total of 0.473% calcium and 0.347% phosphorus. Salt mixture 12 was so constructed that 4% of the mixture would supply approximately this amount of phosphorus (0.334%) and slightly more calcium (0.576%).

All the salts used in the preparation of the mixture for the following studies were of either reagent or c.p. quality. The salts which are present in small quantities were weighed on an analytical balance and, with the exception of the iodide, were ground together in a mortar and worked into the sodium chloride. Free iodine was liberated if the iodide was included with these salts, so it was worked into the sodium chloride separately. Due to the excess of basic over acidic radicals the tendency to lose iodine from the final mixture is reduced, but in cases where a very high degree of purity is not required a stabilized form of iodide such as that suggested by Johnson and Frederick ('40) could be used. The final salt mixture was ground and passed through a 40-mesh sieve.

The low-phosphorus basal diet [3] used for the comparison of the two salts (salts no. 12 and salts no. 351) was composed of the following ingredients expressed in per cent: Alcohol extracted fibrin 18, lard 10, dextrinized starch 67, agar 2, liver extract 2 [4] and glucose [5] containing 20 mg. of thiamine chloride [6] per 100 gm., 1. Two-tenths milliliter of cod liver oil was given to each animal from a tuberculin syringe three times per week. This diet contains from 0.02 to 0.03% of both calcium and phosphorus. Each salt mixture was fed at three levels — 2, 3 or 4 gm. per 100 gm. of basal diet.

Rats were used as experimental animals. Shortly after birth, usually on the second day, the litter of young rats was reduced to six. Only those litters were used in which it was possible to have all six remaining animals of the same sex. At 25 days of age the animals were placed on experiment.

[3] Based on previous reports from this laboratory (Jones, '38, '39).

[4] Liver fraction E supplied by Dr. C. E. Graham of The Wilson Laboratories, Chicago, Illinois.

[5] Cerelose.

[6] Supplied by Merck and Co., Inc., Rahway, N. J.

A total of sixteen litters was used for this comparison and the litter-mates were distributed so that each dietary group included one animal of each litter. This gave identical distribution among the various groups of animals in regard to litters and sex. Without exception the experimental diets were given for 28 days.

At the conclusion of the feeding period the animals of some of the litters were bled under ether anesthesia, and the sera analyzed for calcium by the method of Clark and Collip ('25) and for inorganic phosphorus by the method of Gunther and Greenberg ('29). The remaining animals were chloroformed. The right femurs of all animals were removed and the percentage ash determined on the lipid-free bones. The results of the determinations on the sera and femurs are given as averages for each group in the first section of table 2. Both the absolute and relative amounts of femur ash are recorded. In addition the initial body weight and gain in weight for each group are given.

From the data given in table 2 it is clear that at all levels salt mixture 12 was more effective in producing growth and calcification and in maintaining normal amounts of calcium and inorganic phosphorus in the serum than was salt mixture 351 at the corresponding level. Not only is there a definite difference in the averages when one mixture is compared with the other at the same level, but judged by either the absolute or relative amounts of femur ash, there was not a single animal receiving salts no. 351 which showed as much calcification as its litter-mate receiving an equal amount of salts no. 12. With two exceptions the same was true for gain in body weight. The calcium of the serum was higher and the inorganic phosphorus lower at all levels of salts no. 351 than at any level of salts no. 12, reflecting the difference in ratio as well as absolute amounts of calcium and phosphorus in the salt mixture. With salts no. 351 the calcium was definitely above and the inorganic phosphorus definitely below normal.

TABLE 2

The effect of various salt mixtures on calcification and concentrations of calcium and phosphorus in blood serum.

GROUP	SALT MIXTURE	AMOUNT OF SALT MIXTURE	INITIAL WEIGHT OF ANIMALS	GAIN IN WEIGHT OF ANIMALS	ASH OF FEMURS	SERUM			
						Number of animals	Number of determinations	Ca	P
I. Comparison of salt mixtures 351 and 12 on a basal diet low in phosphorus									
						16 animals in each group			
no.	*no.*	*%*	*gm.*	*gm.*[1]	*mg.*[1]	*%* [1]			*mg./100 cc.*[1]	*mg./100 cc.*[1]
1	351	2	50.6	58.5 ± 6.1	53.7 ± 3.2	38.7 ± 4.5	8	6	14.66 ± 0.19	6.87 ± 0.35
2	12	2	50.7	94.1 ± 5.4	99.3 ± 4.4	49.8 ± 0.79	6	5	12.60 ± 0.13	8.11 ± 0.53
3	351	3	50.8	68.3 ± 3.9	78.4 ± 4.1	46.0 ± 0.91	8	6	14.20 ± 0.33	7.42 ± 0.55
4	12	3	50.7	100.4 ± 4.4	130.2 ± 7.6	54.4 ± 0.26	8	6	12.30 ± 0.22	9.15 ± 0.45
5	351	4	50.7	75.9 ± 6.5	98.1 ± 3.9	49.9 ± 0.70	8	6	13.96 ± 0.36	7.45 ± 0.29
6	12	4	50.7	102.1 ± 2.4	138.1 ± 2.9	55.7 ± 0.41	8	6	12.36 ± 0.14	9.19 ± 0.38
II. Comparison of salt mixtures 351 and 12 on a basal diet high in phosphorus										
						14 animals in each group				
7	351	2	53.8	123.2 ± 4.4	146.5 ± 2.3	55.3 ± 0.40 [2]	7	7	11.99 ± 0.19	9.85 ± 0.47
8	12	2	53.9	118.4 ± 5.9	132.4 ± 2.3 [3]	54.1 ± 0.40 [2]	7	7	11.30 ± 0.23	10.30 ± 0.22
9	351	3	53.9	121.3 ± 6.2	147.8 ± 4.8	55.2 ± 0.61 [2]	7	7	12.23 ± 0.22	7.92 ± 0.19
10	12	3	54.5	124.8 ± 6.4 [2]	146.3 ± 3.6 [2]	55.6 ± 1.2 [2]	7	7	11.61 ± 0.16	10.24 ± 0.33
11	351	4	53.8	117.0 ± 5.3	148.2 ± 3.2	55.3 ± 0.45	7	7	13.01 ± 0.23	8.59 ± 0.55
12	12	4	53.9	121.0 ± 6.2	147.9 ± 4.2	55.7 ± 0.48	7	7	12.09 ± 0.19	10.12 ± 0.39
III. Comparison of salt mixtures 12, 14 and 14 + 0.25% phosphorus on a basal diet low in phosphorus										
						12 animals in each group				
13	12	4	52.1	89.5 ± 2.5	135.4 ± 4.2	55.3 ± 0.46	8	7	12.26 ± 0.23	8.38 ± 0.35
14	14	4	52.1	89.6 ± 5.2	133.4 ± 3.3	54.4 ± 0.37	8	7	11.67 ± 0.38	8.51 ± 0.58
15	14+ 0.25% P	4	52.1	82.0 ± 5.0	122.3 ± 2.3	54.3 ± 0.41	8	7	12.13 ± 0.11	9.08 ± 0.34

[1] Standard error of the mean follows the average.

[2] Average of thirteen animals.

[3] Average of twelve animals.

It is clear from the above results that salt mixture 351 is not satisfactory for use with a basal diet containing negligible amounts of phosphorus even when the mixture is fed in amounts of 4 gm. per 100 gm. of basal diet. On the other hand, the feeding of a salt mixture of higher phosphorus and lower calcium content in amounts of either 3 or 4 gm. per 100 gm. of the same basal diet produces much better calcification and allows for greater growth. These results are in agreement with those of Shohl ('36) who showed that a diet containing calcium and phosphorus in the amounts furnished by 2 to 4 gm. of salts no. 351 per 100 gm. of basal diet and no other source of phosphorus would be definitely rachitogenic for the rat.

After finding that salt mixture 12 was more satisfactory than salts no. 351 for use with a basal diet low in phosphorus, the two mixtures were then compared with each other when used with a basal diet containing liberal amounts of phosphorus. For this purpose the following diet expressed in per cent was used: crude casein 18,[7] dried brewer's yeast 6,[8] agar 2, lard 10, and dextrinized starch 64. Cod liver oil was given as above. The two salt mixtures were again fed at levels of 2, 3 and 4 gm. per 100 gm. of the basal diet. The same technique was followed with regard to choice and distribution of animals and experimental procedure. Fourteen litters of animals were used and calcium and phosphorus were determined on the sera of seven individual animals from each group.

The results of this comparison are given in the second part of table 2. Judged by any of the criteria, there is very little difference between the two salt mixtures. At the 2-gm. level, salts no. 351 appears to be a little superior to salts no. 12 both in producing calcification and growth. At the other levels the two mixtures are about equal. Although not very great, there is a constant difference in serum calcium and phosphorus. As in the first series of experiments, calcium was

[7] Furnished by Smith, Kline and French, Inc., Philadelphia, Pa.
[8] Supplied by Anheuser-Busch, Inc., St. Louis, Mo.

greater and the inorganic phosphorus was less in those animals receiving salts no. 351 than in those given salt mixture 12. There was less variation in the degree of calcification among the several groups in the latter, than in the first, series of experiments. This was particularly true with salts no. 351 which emphasizes the necessity of having a liberal amount of phosphorus in the basal diet if this mixture is to be used successfully.

The animals receiving the high-phosphorus basal diet grew more with either salt mixture and at all levels than any of the groups of animals receiving the low-phosphorus basal diet. This might be interpreted as indicating that even with salts no. 12 at the 4-gm. level there was still a deficiency of phosphorus. In view of this difference a direct comparison was made between the low-phosphorus (fibrin and liver extract) diet and the high-phosphorus (casein and yeast) diet with 4 gm. of salt mixture 12 added to 100 gm. of each diet. Seven animals comprised each group. Here again growth was definitely better on the diet containing the higher amount of phosphorus, but there was little difference in calcification.

In another series of experiments the effect of adding phosphorus as monopotassium phosphate to the low-phosphorus diet was studied. The extra phosphorus increased calcification when only 2 gm. of salts 12 per 100 gm. of basal diet were fed, but not when the larger amounts of the salt mixture were given.

As a final experiment, salts no. 12 were compared with salt mixture no. 14. The latter mixture is the same as salts no. 12 except that 4 moles of the calcium carbonate were replaced with an equal number of moles of dicalcium phosphate ($CaHPO_4$). This increases the phosphorus considerably without appreciably altering the relative amounts of the other constituents. When fed at a level of 4% of the diet, salt mixture 14 supplies 0.57% calcium and 0.55% phosphorus with a calcium to phosphorus ratio of 1.04. When a high phosphorus mixture like salts no. 14 is used with a high-phosphorus basal diet such as the casein-yeast diets, there may be danger of

supplying too much phosphorus in comparison with the calcium. Therefore, in addition to comparing salt mixtures 12 and 14 at the 4-gm. level, another group of animals was given the same amount of salts no. 14 plus an additional 0.25% phosphorus as disodium phosphate. This additional phosphorus is approximately the amount supplied by 18% of casein and 6% of yeast which are so frequently used in synthetic diets. The calcium to phosphorus ratio of such a diet is 0.71. The results of this experiment are summarized in the third section of table 2. Here again growth was not so good as on the casein-yeast diet, but in this experiment there was a larger proportion of females than in any of the other series. It does, however, seem that regardless of the amount of phosphorus added, growth on the low-phosphorus basal diet was somewhat below that on the high-phosphorus diet. This is probably due to an insufficiency of some members of the vitamin B complex. The levels of serum calcium and inorganic phosphorus are about the same in all three groups and there is practically no difference in the percentage of femur ash. Growth on the diet containing the largest amount of phosphorus was slightly below that of the other two groups, as was also the weight of femur ash. It seems that this high amount of phosphorus and rather low calcium to phosphorus ratio may have slightly inhibited growth and calcification. If this is the case salt mixture 14 would not be so satisfactory as salts no. 12 when used with a basal diet containing a liberal amount of phosphorus, but salt mixture 12 appears to produce equally good results as salts no. 14 when used with a basal diet low in phosphorus. It seems safe to conclude that 4 gm. of salt mixture 12 per 100 gm. of basal diet and in the presence of ample vitamin D produces excellent calcification on either a low-phosphorus or a high-phosphorus basal diet. We have also used this salt mixture with apparent success for reproduction studies in mice on both low-phosphorus and high-phosphorus basal diets.

SUMMARY

The composition of a complete salt mixture (salt mixture 12) satisfactory for use with purified basal diets either low or high in phosphorus is given. This mixture has been compared with salt mixture 351 of Hubbell, Mendel and Wakeman at levels of 2, 3 and 4 gm. per 100 gm. of basal diet. With a basal diet low in phosphorus, the new salt mixture was definitely superior to salt mixture 351 for both calcification and growth at all levels fed. With a basal diet containing liberal amounts of phosphorus, 2 gm. of salt mixture 351 were slightly better than an equal amount of salt mixture 12, but at the 3- and 4-gm. levels there was no detectable difference. When fed at either 3 or 4 gm. and in the presence of liberal amounts of vitamin D the salt mixture described gives satisfactory results with either a phosphorus-high or a phosphorus-low basal diet. In the latter case, however, 3 gm. per 100 gm. of basal diet appears to be a border-line amount and under these conditions the feeding of 4 gm. of the mixture is recommended. At this level the salt mixture described gives very satisfactory results regardless of the amount of phosphorus in the basal diet.

LITERATURE CITED

BETHKE, R. M., C. H. KICK AND W. WILDER 1932 The effect of the calcium-phosphorus relationship on growth, calcification, and blood composition of the rat. J. Biol. Chem., vol. 98, p. 389.

CLARK, E. P., AND J. B. COLLIP 1925 A study of the Tisdall method for the determination of blood serum calcium with a suggested modification. J. Biol. Chem., vol. 63, p. 461.

DANIELS, A. L., AND G. J. EVERSON 1935 The relation of manganese to congenital debility. J. Nutrition, vol. 9, p. 191.

EVANS, R. J., AND P. H. PHILLIPS 1939 A new low fluorine diet and its effect upon the rat. J. Nutrition, vol. 18, p. 353.

GUNTHER, L., AND D. M. GREENBERG 1929 A note on the determination of the inorganic phosphate of the serum on the filtrate from calcium analysis. J. Biol. Chem., vol. 82, p. 551.

HUBBELL, R. B., L. B. MENDEL AND A. J. WAKEMAN 1937 A new salt mixture for use in experimental diets. J. Nutrition, vol. 14, p. 273.

JOHNSON, F. F., AND E. R. FREDERICK 1940 Stabilization of iodine in salt and feedstuffs. Science, vol. 92, p. 315.

JONES, J. H. 1938 The use of fibrin in synthetic diets. J. Nutrition, vol. 15, p. 269.

———— 1939 A study of rachitogenic diets composed of purified food materials. J. Nutrition, vol. 17, p. 601.

PHILLIPS, P. H., AND E. B. HART 1935 The effect of organic dietary constituents upon chronic fluorine toxicosis in the rat. J. Biol. Chem., vol. 109, p. 657.

RICHARDSON, L. R., AND A. G. HOGAN 1940 Adequacy of a milk diet for the rat. J. Nutrition, vol. 19, p. 13.

SHOHL, A. T. 1936 Rickets in rats. XV. The effect of low calcium-high phosphorus diets at various levels and ratios upon the production of rickets and tetany. J. Nutrition, vol. 11, p. 275.

SURE, B. 1940 The essential nature of choline for lactation and growth of the albino rat. J. Nutrition, vol. 19, p. 71.

———— 1941 Dietary requirements for fertility and lactation. XXIX. The existence of a new dietary factor essential for lactation. J. Nutrition, vol. 22, p. 499.

WAISMAN, H. A., O. MICKELSEN, J. M. MCKIBBIN AND C. A. ELVEHJEM 1940 Nicotinic acid potency of food materials and certain chemical compounds. J. Nutrition, vol. 19, p. 483.

THE SULFUR METABOLISM OF CHILDREN *

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FOUR FIGURES

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Information relating to sulfur metabolism in growing children has been limited. Schwarz ('10) has presented detailed data for a 5-year-old child. Folin ('05) in his classic paper and, later, Clark ('26) explored the over-all sulfur economy and urinary excretion in adults. An opportunity to examine the sulfur retention, excretion and urinary sulfur partition of the normal child, in relation to nitrogen metabolism, was presented during a detailed, continuous, 45-day metabolic balance study of eight children, 8 to 12 years of age. The results contribute information concerning the manner in which sulfur is metabolized and retained by children.

EXPERIMENTAL

The subjects studied were normal, healthy children [1] as judged by past nutritional history, hematological observations, growth rate and detailed x-ray studies of skeletal maturity. Seven of the subjects were boys; one girl, subject III, was

* Some of the data in this paper were included in a report given before the Division of Biological Chemistry of the American Chemical Society at the national meeting in Cincinnati, Ohio, April 8–12, 1940.

[1] One child, subject I, deviated from normal only in having an essential idiopathic lipemia (Bernstein, Williams, Hummel, Shepherd and Erickson, '39). Although originally not under study for interpretation with the normal children, it was found that this subject fitted into the general picture of chemical growth presented by the other children, and therefore he has been included in the normal group for the purposes of the present discussion.

included in the group. Throughout the study, the children lived at the Methodist Children's Village, Detroit, under conditions of healthful activity and emotional security, the only conditions under which observations of normal metabolism are possible.

The diet was made up of twenty-two common foods, given daily and in constant quantity for each child throughout the study, at a caloric level suitable for normal growth (Macy, '42). The diet furnished adequate supplies of minerals, proteins, vitamins and all known nutritional essentials for physical health and well-being.

The study was divided into 5-day balance periods. Samples of the mixed dietary were analyzed each period, for nitrogen by the Kjeldahl method and for sulfur after combustion of vacuum-dried samples in the Parr oxygen bomb. All samples of urine were collected quantitatively and held, under toluene, in a refrigerator until the 5-day collection was complete. Carmine was given at the end of each period, to mark the feces of separation. Urinary sulfur partitions were obtained by the methods of Folin ('05–'06) and Denis ('10–'11). Sulfur partition studies were instituted following two 5-day periods which allowed time both for some physiological adjustment to the experimental regimen and for establishing the metabolic routine so that accuracy of the collecting technique was assured. During the subsequent nine 5-day periods, composite samples of urine were used to determine the sulfur partition.

In table 1 the average daily values for each of the individuals are presented, with their standard deviations. The data recorded are the means of averages of duplicate or triplicate analyses of samples collected within the 5-day periods and carry considerably more weight than single, isolated determinations. Errors inherent in the method of determining the sulfur partition, particularly with neutral and ethereal sulfate, tend to be compensated in the mean values. In table 1 the subjects are arranged in order of increasing age, which is also the sequence of increasing body weight.

TABLE 1

Average daily nitrogen and sulfur balances and urinary sulfur partition during 45 consecutive days.[1]

	I	II	III	IV	V	VI	VII	VIII
Age—5th period, months	103	104	112	114	127	132	143	147
Body weight—5th period, kg.	24.49	26.94	28.32	29.12	31.84	33.70	35.82	41.85
Total weight gain—1st–9th period, kg.	1.28	0.30	2.89	—0.96	1.01	0.65	1.10	0.92
Recumbent length—7th period, cm.	120.4	132.2	131.3	140.9	139.8	145.2	138.3	159.3
Basal heat production								
Total (Cal./hr.)	54.0	51.4	54.5	60.9	56.1	57.1	55.7	59.8
Cal./sq.m./hr.	61.4	51.1	54.0	56.2	50.1	48.2	47.4	43.4
Lazation rate (defecations per day)	1.3	1.2	1.1	1.7	1.0	0.9	1.0	0.7
	±0.2	±0.2	±0.2	±0.3	±0.1	±0.3	±0.2	±0.2
AVERAGE PER DAY								
Intake								
Nitrogen, gm.	13.17	13.17	13.17	13.17	13.38	13.38	13.38	13.38
	±.22	±.22	±.22	±.22	±.22	±.22	±.22	±.22
Sulfur, mg.	977	977	977	977	1003	1003	1003	1003
	±58	±58	±58	±58	±58	±58	±58	±58
Ratio nitrogen/sulfur [2]	13.5	13.5	13.5	13.5	13.3	13.3	13.3	13.3
Fecal outgo								
Nitrogen, gm.	1.43	1.12	1.19	1.54	1.15	1.21	1.43	1.45
	±.03	±.11	±.07	±.16	±.07	±.23	±.09	±.14
Sulfur, mg.	135	106	118	145	115	113	133	138
	±9	±10	±7	±8	±10	±16	±11	±10
Ratio nitrogen/sulfur [2]	10.6	10.5	10.1	10.6	10.0	10.7	10.7	10.5
Absorption [3]								
Nitrogen, gm.	11.74	12.05	11.98	11.63	12.23	12.17	11.95	11.93
Per cent of intake	89.1	91.5	91.0	88.3	91.4	91.0	89.3	89.2
Sulfur, mg.	842	871	859	832	888	890	870	865
Per cent of intake	86.2	89.2	87.9	85.2	88.5	88.7	86.7	86.2
Ratio nitrogen/sulfur [2]	13.9	13.8	13.9	14.0	13.8	13.7	13.7	13.8
Urinary outgo								
Nitrogen, gm.	10.96	11.26	10.55	10.82	11.10	11.21	11.02	11.02
	±.37	±.39	±.47	±.45	±.12	±.19	±.43	±.37
Sulfur, mg.	750	784	726	746	773	793	776	754
	±23	±26	±25	±28	±26	±18	±23	±20
Ratio nitrogen/sulfur [2]	14.6	14.4	14.5	14.5	14.4	14.1	14.2	14.6
Retention								
Nitrogen, gm.	0.78	0.79	1.43	0.81	1.13	0.96	0.93	0.91
	±.16	±.10	±.29	±.28	±.30	±.21	±.30	±.10
Sulfur, mg.	92	87	133	86	115	97	94	111
	±65	±57	±66	±63	±59	±68	±75	±66
Ratio nitrogen/sulfur [2]	8.5	9.0	10.8	9.4	9.9	9.9	9.9	8.2
Urinary sulfur partition								
Inorganic SO_4, mg.	655	676	622	620	644	664	673	604
	±15	±15	±23	±21	±17	±16	±19	±16
Ethereal SO_4, mg.	43	42	39	42	50	54	43	68
	±10	±6	±4	±7	±5	±8	±4	±4
Neutral sulfur, mg.	52	66	65	84	79	75	60	82
	±16	±8	±11	±16	±12	±11	±6	±14
Per cent of total urinary sulfur								
Inorganic SO_4	87.4	86.2	85.7	83.1	83.3	83.7	86.8	80.1
Ethereal SO_4	5.7	5.4	5.4	5.6	6.5	6.8	5.5	9.0
Neutral sulfur	6.9	8.4	8.9	11.3	10.2	9.5	7.7	10.9

[1] Values given are means or means plus or minus standard deviations.
[2] Ratios were calculated from milligrams of nitrogen and sulfur.
[3] Calculated as the intake minus fecal outgo of each element.

Intake and absorption

The four younger children received daily a diet containing 0.973 ± 0.043 gm. sulfur and 13.09 ± 0.39 gm. nitrogen, while the older children, who received a similar diet containing additional calories in the form of potato and washed sweet butter, consumed 0.999 ± 0.037 gm. sulfur and 13.27 ± 0.48 gm. nitrogen. Thus, the diets were designed to satisfy the needs of the subjects for all nutriments, but were almost identical in the quantities of nitrogen and sulfur furnished.

With the metabolic balance technique it is not possible to determine the actual amount of sulfur or nitrogen absorbed by the body. This difficulty arises because of the complex nature of the fecal losses. How much fecal nitrogen and sulfur, if any, was absorbed and re-excreted into the intestine is impossible to determine. In metabolic procedure the closest indication of nitrogen and sulfur absorption is obtained by calculation of the intake minus fecal outgo.

The per cent absorption of nitrogen was consistently greater than that of sulfur, resulting in a slightly higher nitrogen-to-sulfur ratio for the absorption than for the intake. Whether or not this was due to either preferential utilization of nitrogen on the part of the body, an excretion of sulfur into the intestine, or the seizure of sulfur compounds by intestinal flora, is not known. There is remarkable constancy among the nitrogen-to-sulfur ratios for the absorption. A fairly strict parallelism of the nitrogen and sulfur absorption is evident in figure 1, in which the per cent of nitrogen intake absorbed is plotted against the corresponding values for sulfur.

Excretion of nitrogen and sulfur in urine and feces

In all phases of metabolism there appear to be parallelisms between nitrogen and sulfur. They occur together in the proteins of the foods and are stored together in the body proteins. While there are many compounds of significance in body economy which contain only one or the other of these elements, the great preponderance of both nitrogen and sulfur storage goes into the construction of proteins.

Most of the dietary nitrogen and sulfur lost from the body appears in the urine. The urinary sulfur loss in our subjects averaged 77.0% of the intake and the urinary nitrogen loss was 82.8% of the intake. The feces, on the other hand, contained only 12.7% and 9.9%, respectively, of the sulfur and nitrogen intakes. Thus, the urine contains five to seven times as much sulfur and seven to ten times as much nitrogen as do the

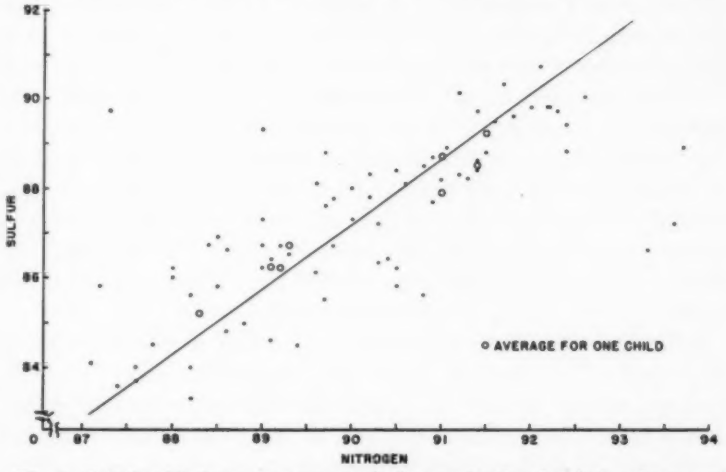

Fig. 1 Relationship between per cent of nitrogen intake absorbed and per cent of sulfur intake absorbed. Dots represent averages for single subjects for 5-day periods; circles are the means of nine 5-day periods for each child.

feces. This is essentially in agreement with observations on adult men (Clark, '26).

The nitrogen-to-sulfur ratios for the urine averaged 14.4; for the feces, 10.5; for the dietary, 13.4. This shift in the nitrogen-to-sulfur ratio during passage of the residue through the alimentary canal is the result of the total of the processes of gastrointestinal secretion, excretion, absorption and bacterial growth which result in the complex fecal residue.

Urinary sulfur partition

Inorganic sulfate. By far the greatest quantity of the sulfur lost in urine is in the form of inorganic sulfates. The children

in this study excreted an average of 84% of their total urinary sulfur in this form. As many have pointed out, the percentage lost as inorganic sulfate is variable, dependent primarily on the level of sulfur intake. On the other hand, ethereal and neutral sulfur are influenced less by diet and tend to remain more nearly constant.

Ethereal sulfur. Urinary ethereal sulfate has been studied with interest because of its relation to toxic compounds. The compounds comprising this group are indole, skatole and phenol, and perhaps other substances conjugated with sulfuric acid and excreted as the potassium salt. These residues are believed to arise from the breakdown of the corresponding amino acids, tryptophane, phenylalanine and tyrosine. Studies have indicated that the source of these toxic substances is twofold: from some normal, minor, but constant metabolic processes, and from putrefactive action of the bacteria in the intestine. The liver has been shown by Pelkan and Whipple ('22) to be the site of this detoxication process.

Indican is the ethereal sulfate of indole. Folin has shown that, while the level of indole sulfuric acid in the urine is perhaps a fair index of the extent to which intestinal putrefaction is taking place, there is poor correlation of total ethereal sulfate sulfur of the urine with urinary indican levels. Dubin ('16) has found that in cases of severe intestinal obstruction and other disturbances of normal protein digestion there is an increase in both free and conjugated phenols, and suggests that total phenol excretion can be used as a measure of intestinal putrefaction. In Folin's studies ('05) the ethereal sulfate level in the urine of adults consuming a mixed dietary was found to range from about 60 to 100 mg. per day. Schwarz ('10) found in the 5-year-old child an average of 23 mg. per day. The ethereal sulfate excretion by our 8- to 12-year-old group averaged 48 mg., which is a level between the adult and the younger child and indicates a graded output of ethereal sulfate, increasing with age and perhaps influenced by intensity of protein metabolism. An examination of the individual values from our study reveals that the younger children excreted ethereal sulfate at a slightly lower level than did the older

ones, although they consumed essentially the same quantity of protein.

A marked deviation with age from the slow upward trend of ethereal sulfate is seen in subject VIII, and is suggestive that growth, age or level of protein intake are not the only factors determining urinary ethereal sulfate excretion. In looking for factors to explain this deviation further, we have

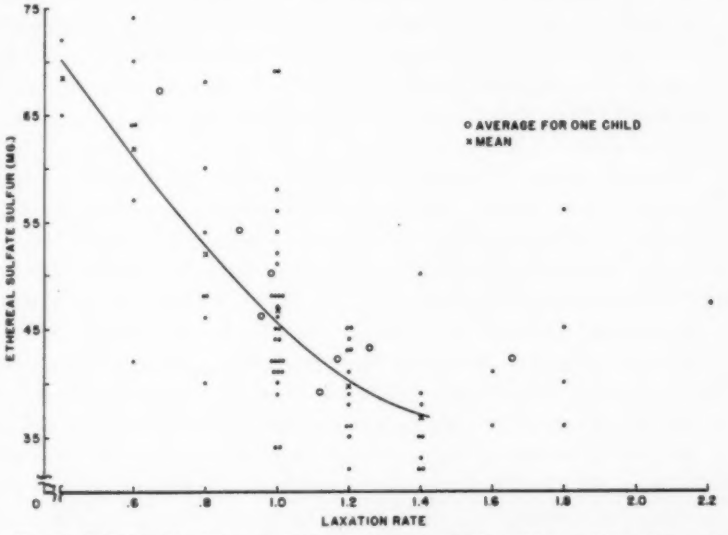

Fig. 2 Relationship between urinary ethereal sulfate sulfur and laxation rate (bowel movements per day). Dots represent averages for single subjects for 5-day periods; circles are the means of nine 5-day periods for each child; crosses are the means for all subjects showing a given laxation rate.

recorded the average laxation rate (bowel movements per day) of each child, and with increasing age there was generally a decreasing laxation rate, although subject IV, in the middle-age group, deviates from this general trend in having a high laxation rate. In figure 2 the urinary ethereal sulfate is plotted against laxation rate and shows the upward trend in ethereal sulfate, with lower laxation rates. It is possible that a low laxation rate with longer retention of feces may be accompanied by a considerable increase in the ethereal sulfate

fraction of the urine, owing to more extensive intestinal putrefaction.

A study of laxation rate, ethereal sulfate excretion and urinary indican should be carried out on enough subjects of sufficiently widely varying laxation rates to provide an adequate test for the relationships which may exist between these physiologic manifestations. That intestinal flora acts more extensively on the fecal residue in subjects of low laxation rate is demonstrated by studies in this laboratory (unpublished data), in which the amounts of food cellulose and hemicellulose disappearing during passage through the gastrointestinal tract were found to be an inverse function of the laxation rate.

Neutral sulfur. Neutral sulfur is the fraction of total urinary sulfur which cannot be accounted for as either inorganic or ethereal sulfate sulfur. Inasmuch as the value is obtained by difference and is normally only a small fraction of the total urinary sulfur, the individual determinations tend to be rather unreliable. During the last 5-day period of observation with our subjects, the urinary sulfur partition was studied daily. Wide fluctuations occurred in the neutral sulfur values for each individual and each child varied within a more or less characteristic range. Undoubtedly, some of the variation was due to errors inherent in the method of analysis (Folin, '05); therefore, it is important to obtain a number of observations sufficient for averaging before interpretation is undertaken. Folin suggested that the neutral sulfur fraction reflects constant endogenous metabolic phenomena and demonstrated that it is little influenced by ordinary changes in dietary protein levels. Brody et al. ('34) point out the observation of Amann and Mourot that an increase in urinary neutral sulfur excretion can be produced when the protein intake is increased manyfold above the normal level.

The neutral sulfur fraction is made up of a variety of organic sulfur compounds which normally appear in small amounts in the urine. Among these is cystine, which according to Medes ('36) in the normal subject may contribute as much as 9 mg. of sulfur daily. A portion of the neutral sulfur also may be accounted for as the sulfur of the urinary pig-

ments and thiosulfates, thiocyanates, taurine, mercaptans, and mercapturic acid derivatives. The fraction is of a complex nature and little is known of the forms of sulfur which constitute it.

The average daily level of neutral sulfur output in the age group 8 to 12 years (table 1) was 70 mg., which is higher than the 45 mg. value reported by Schwarz ('10) for the 5-year-old child and is in agreement with Brody's (Brody, Procter and Ashworth, '34) prediction tables for subjects in the weight range studied. The range of results in normal adults, according to Folin ('05), was 52 to 76 mg. daily.

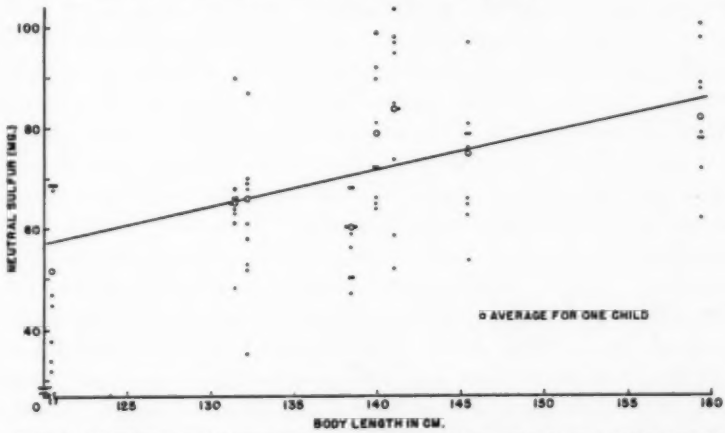

Fig. 3 Relationship between urinary neutral sulfur output and body length. Dots represent averages for single subjects for 5-day periods; circles are the means of nine 5-day periods for each child.

Our observations with the eight children show not more than a remote relationship between neutral sulfur output and age. Brody et al. ('34), studying a variety of animals weighing from 0.02 kg. to 1000 kg., demonstrated a power relationship between body weight and neutral sulfur output. It is not surprising, however, with a limited number of subjects within a restricted weight range, to find variations between neutral sulfur and body weight which may represent only normal deviations within a broad general law of relationship.

Figure 3 shows that there was a tendency in our subjects toward relationship between urinary neutral sulfur and re-

cumbent body length. In contrast, there was a lack of relationship with either body weight or surface area. It is of interest to recall that Daniels (Daniels and Hejinian, '29) has suggested the use of a creatinine-height coefficient, for there is greater constancy of creatinine output per unit height than per unit body weight. While body length and neutral sulfur output may be linked directly, or through interrelation with some common factors, the urinary neutral sulfur is undoubtedly influenced by many other, more complex factors which determine the individual's whole metabolic performance.

Inasmuch as urinary neutral sulfur excretion may reflect endogenous metabolic performance, various measures such as basal heat production, body surface, and creatinine output might be expected to correlate well with level of neutral sulfur. In the present study the relationship is not clearly defined; however, the general upward trend of neutral sulfur excretion with the indices of increased scale of metabolism is undeniable. Basal heat output shows some variations which coincide with those of neutral sulfur. Subject IV had a high basal heat production for his age, equal to that of subject VIII, who was 3 years his senior and 12.5 kg. heavier. Accompanying the equivalent heat production, the two children had the same neutral sulfur output in their urine. On the other hand, subject VII was short and had a somewhat low basal heat output for his size, with a rather low neutral sulfur output. The results for this urinary fraction illustrate the complexity of the various mechanisms which contribute to the end results of sulfur metabolism.

Sulfur and nitrogen retention

The levels of nitrogen and sulfur retention for the children were not related to age or body size. The greatest retention of these elements occurred in subjects III and V, in the middle of the group, with reference to age and body weight. There seems to be no constant relationship between weight gains and retention values, although the most rapidly growing subject, III, showed the greatest sulfur and nitrogen re-

tentions. The frequent lack of relationship between various indices of growth serves to re-emphasize the fact that no single measurement of a physical attribute or phase of chemical growth over a limited period of time is a reliable criterion for judging the rate at which the complex process of growth and development is progressing.

The greater portions of the nitrogen and sulfur stored by the growing organism are simultaneously incorporated into body protein. For this reason, it is to be expected that their

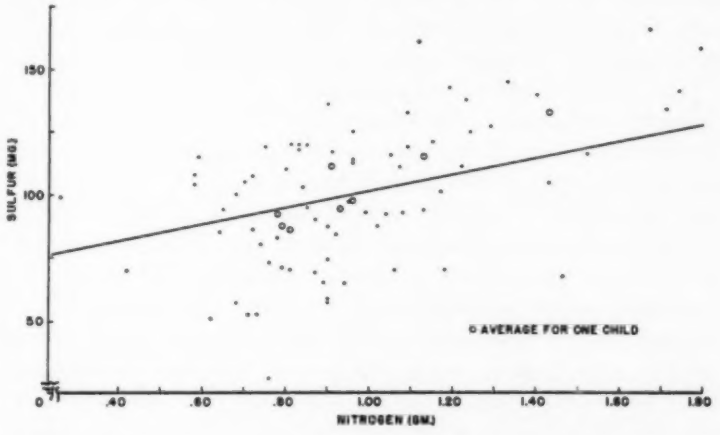

Fig. 4 Relationship between sulfur retention and nitrogen retention. Dots represent averages for single subjects for 5-day periods; circles are the means of nine 5-day periods for each child.

retention would run almost parallel. That this is true to some extent is shown in figure 4, in which retention of nitrogen is plotted against that of sulfur, but the variabilities are more evident from study of the nitrogen-to-sulfur ratios of the retentions (table 1). This ratio varied from 8.2 to 10.8 and averaged 8.8.

Retention is determined in a metabolic balance study as the intake of an element minus the sum of urinary and fecal outgo. Observed retention and true retention are equal only when urine and feces are the only routes by which an element is

lost to the body. In the dog, essentially a non-sweating animal, this would be nearly true. In the human subject, losses of some substances through the skin may be highly significant; this has been shown by Swanson and Iob ('33) with infants. However, in the classical metabolic balance these losses would be included in the retention value. Observations on growing dogs have revealed that the nitrogen-to-sulfur retention ratio is commonly 14.5, and Lewis ('16) has shown during fasting and consequent tissue catabolism a urinary nitrogen-to-sulfur ratio of 13 to 16. In the studies of Deuel et al. (Deuel, Sandiford, Sandiford and Boothby, '28), in one human subject the urinary nitrogen-to-sulfur ratio varied from 5 to 50, over a long period of protein deprivation.

The few analyses available on the nitrogen and sulfur content of the whole body, summarized by Shohl ('29), reveal that newborn infants have a nitrogen-to-sulfur ratio of 8.7, while in adults it is 18.8. Studies in this laboratory, of the nitrogen and sulfur contents of both mixed muscle protein and the protein residues of many soft organs, have shown a nitrogen-to-sulfur ratio of about 15. There are points of concentration of nitrogen and sulfur in the body other than muscle and soft organs which might result in an over-all nitrogen-to-sulfur ratio other than 15 for the whole body. However, Peters and Van Slyke ('31) state that "no matter what may be the ratio of N:S in the dietary protein, the proportions of the two elements retained in the body tend to remain about 14.5:1, the ratio characteristic of animal protein."

Little has been done on the losses of substances through the skin of human subjects. McCance ('36) has shown that sodium chloride losses through the skin during heavy perspiration may cause severe depletion of sodium chloride from the body. Swanson and Iob ('33) revealed that skin losses of mineral elements by the human infant, particularly with reference to potassium, are highly significant even under normal conditions of perspiration and must be measured if true storage of such elements is to be accurately estimated by the metabolic balance technique. More recently, Freyberg and Grant ('37)

determined the skin losses of two adults during periods in which no visible sweating took place. Under these conditions the subjects lost an average of 88 mg. of sulfate sulfur and 200 mg. of soluble nitrogen per 24 hours. The average surface area of the subjects [2] was 1.93 sq.m., which would indicate an average daily loss of 46 mg. of sulfur and 104 mg. of nitrogen per square meter of body surface.

Applying the above results to the group of children in the present study, our subjects may have lost through the skin from 41 to 63 mg. of sulfate sulfur and from 93 to 144 mg. of soluble nitrogen every 24 hours. The observed sulfur retentions varied from 86 to 133 mg. per 24 hours. If the calculated skin losses are correct they represent nearly 50% of the retention value for sulfur. On the other hand, the possible skin loss of nitrogen is of less significance in a retention of 780 to 1130 mg. If these assumptions are correct, therefore, the ratio of nitrogen to sulfur in the true retention of our children may have been considerably higher than the observed retention would indicate. Perhaps future investigations of skin excretion will clarify the interpretation of the results of metabolic studies on these and other elements.

SUMMARY

During continuous metabolic balance studies of eight normal children, 8 to 12 years old, over 45 days, under controlled conditions of healthful activity and emotional security, sulfur excretion and retention and urinary sulfur partition were studied in relation to nitrogen metabolism. The elimination in the urine represented 77.0 and 82.8% of the sulfur and nitrogen intakes, respectively. Inorganic sulfur composed 84% of the urinary excretion of that element. The average daily excretion of ethereal sulfur was 48 mg., and of neutral sulfur, 70 mg. With increasing age there was generally a decreasing laxation rate, accompanied by increasing urinary ethereal sulfur. Under the conditions of this experiment, only remote relationships were found between neutral sulfur output and

[2] Personal communication.

age, body weight and surface area, but there was a relation between neutral sulfur and recumbent body length.

The average nitrogen-to-sulfur ratios for intake, feces and urine were 13.4, 10.5, and 14.4, respectively. The nitrogen-to-sulfur ratio of the retentions averaged 8.8; however, if the determined sulfur retentions included losses through the skin comparable to values reported in the literature, the average nitrogen-to-sulfur ratio of the true retentions would be raised to a figure nearer the ratio of 15 which has been indicated for the entire body by analyses of muscle tissue.

LITERATURE CITED

BERNSTEIN, SAMUEL S., HAROLD H. WILLIAMS, FRANCES C. HUMMEL, MARION L. SHEPHERD AND BETTY NIMS ERICKSON 1939 Metabolic observations on a child with essential hyperlipemia. J. Pediatrics, vol. 14, pp. 570–580.

BRODY, S., R. C. PROCTER AND U. S. ASHWORTH 1934 Growth and development with special reference to domestic animals. XXXIV. Basal metabolism, endogenous nitrogen, creatinine and neutral sulphur excretions as functions of body weight. University of Missouri Agricultural Experiment Station Research Bulletin 220.

CLARK, G. W. 1926 Studies in the mineral metabolism of adult man. University of California Publications in Physiology, vol. 5, pp. 195–287.

DANIELS, AMY L., AND LUCEA M. HEJINIAN 1929 Growth in infants from the standpoint of physical measurements and nitrogen metabolism. I. Creatinine. Am. J. Dis. Child., vol. 37, pp. 1128–1134.

DENIS, W. 1910–1911 The determination of total sulphur in urine. J. Biol. Chem., vol. 8, pp. 401–403.

DEUEL, H. J., IRENE SANDIFORD, KATHLEEN SANDIFORD AND W. M. BOOTHBY 1928 A study of the nitrogen minimum. The effect of sixty-three days of a protein-free diet on the nitrogen partition products in the urine and on the heat production. J. Biol. Chem., vol. 76, pp. 391–406.

DUBIN, H. 1916 Physiology of the phenols. J. Biol. Chem., vol. 26, pp. 69–91.

FOLIN, O. 1905 Laws governing the chemical composition of urine. Am. J. Physiol., vol. 13, pp. 66–115.

———— 1905–1906 On sulphate and sulphur determinations. J. Biol. Chem., vol. 1, pp. 131–159.

FREYBERG, R. H., AND R. L. GRANT 1937 Loss of minerals through the skin of normal humans when sweating is avoided. J. Clin. Invest., vol. 16, pp. 729–731.

LEWIS, H. B. 1916 The metabolism of sulfur. I. The relative eliminations of sulfur and nitrogen in the dog in inanition and subsequent feeding. J. Biol. Chem., vol. 26, pp. 61–68.

McCance, R. A. 1936 Experimental sodium chloride deficiency in man. Proc. Roy. Soc. London, Series B, vol. 119, pp. 245–268.

Macy, Icie G. 1942 Nutrition and Chemical Growth in Childhood. Vol. I. Evaluation. Charles C. Thomas, Springfield, Illinois.

Medes, Grace 1936 Metabolism of sulphur. II. The quantitative determination of cystine in normal urine. Biochem. J., vol. 30, pp. 1293–1297.

Pelkan, K. F., and G. H. Whipple 1922 Studies of liver function. III. Phenol conjugation as influenced by liver injury and insufficiency. J. Biol. Chem., vol. 50, pp. 513–526.

Peters, J. P., and D. D. Van Slyke 1931 Quantitative Clinical Chemistry. Vol. I. Interpretations. The Williams and Wilkins Co., Baltimore.

Schwarz, H. 1910 Der Stickstoff- und Schwefelstoffwechsel in Fällen von rachitischem Zwergwuchs und ein Beitrag zum normalen Stoffwechsel eines fünf Jahre alten Knaben. Jahrb. Kinderheilk., vol. 72, pp. 549–574.

Shohl, A. T. 1939 Mineral Metabolism. Reinhold Publishing Co., New York City.

Swanson, W. W., and L. Vivian Iob 1933 Loss of minerals through the skin of infants. Am. J. Dis. Child., vol. 45, pp. 1036–1039.

THE EFFECT OF PANTOTHENIC ACID DEFICIENCY ON THE BLOOD LIPOIDS OF THE DOG

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A marked depression occurs in the concentration of the blood lipoids as the result of pantothenic acid deficiency in the dog. The blood levels are rapidly restored by the administration of the vitamin.

EXPERIMENTAL

Cholesterol and cholesterol esters were determined by Bloor's method (Bloor, '16; Bloor and Knudson, '16). Lipoid phosphorus was determined according to Youngburg and Youngburg ('30) and total lipoids were determined by evaporating to dryness an aliquot of the Bloor filtrate. The residues were extracted with petroleum ether, and the extracts were evaporated and dried to constant weight. These analyses were performed with whole blood in the experiments with puppies, and with plasma in the experiments with the adult dogs. Blood was drawn from fasting animals. Analytical data are given in milligrams per cent.

Twenty-seven dogs (four groups consisting of twelve mongrel puppies, six litter-mate setter puppies, four young adult litter-mate setters and five adult mongrels) were given the same basal ration having a percentage composition of vitamin free casein, 30; dextrose, 41; hydrogenated cottonseed oil,[1] 21; corn oil, 0.15; U.S.P. XI salt mixture, 3.0; bone ash, 2.85 and cod liver oil, 2.0. Control animals were given a

[1] Crisco.

daily supplement consisting of 1 mg. each of thiamine, riboflavin, and pyridoxine, 20 mg. of nicotinic acid, 40 mg. of calcium pantothenate, 200 mg. of choline chloride, and 200 mg. of inositol. Fifty milligrams of α-tocopherol was administered orally every 2 weeks. Additional control animals were given this supplement plus 5 gm. of dried whole beef liver. To produce the deficiency in the test animals, both liver and pantothenic acid were omitted from the supplement. The supplement was included in the diet daily, but when the food intake fell off, the supplement was given by stomach tube.

In general, the dogs on the deficient diet were allowed to eat ad libitum, and the food consumption of the control animals was restricted to the amounts consumed by the deficient animals. The relationships between the test animals, their controls and the food intake is given briefly in the appropriate tables.[2]

Group 1. Six litter-mate setter weanlings (8 weeks old) were placed on their respective diets as shown in table 1. Sixty-seven days after the dogs were placed on the experimental rations, when the rate of growth and the food intake of the deficient animals had fallen markedly, blood samples were drawn and analysed. The deficient animals were then given 4.0 mg. of calcium pantothenate per kilo per day and blood samples were taken and analysed at the time intervals shown in table 1.

Group 2. Nine mongrel weanlings (age 45–57 days) were used as shown in table 2. Forty-two days later, when the growth rate had decreased and the food intake of the deficient dogs had declined, blood samples were taken and analysed. Two of the deficient animals were sacrificed for other studies. The remaining deficient animal was given 4 mg. of calcium pantothenate per kilogram body weight per day in the supplement, and analyses were performed as indicated in table 2.

Group 3. Four male, litter-mate setters (6½ months old) were studied and the changes in the blood lipoids were fol-

[2] Drs. R. H. Silber and A. O. Seeler will discuss this phase of the problem more fully in forthcoming communications.

lowed as the animals continued on their respective diets. Dogs 63 and 73 were given the supplement without pantothenic acid, and were allowed to eat the basal ration ad libitum. The food intake of dog 65 was restricted to that of dog 63, and dog 71 was restricted to what dog 73 ate voluntarily. These control

TABLE 1[1]

Blood lipids of litter-mate puppies in pantothenic acid deficiency.

DATE AND DOG NO.	BASAL DIET	SUPPLEMENT CONTAINED	CHOLESTEROL TOTAL	CHOLESTEROL ESTERS	RATIO OF FREE CHOLESTEROL TO ESTERS	LIPOID P	TOTAL LIPOIDS
3/9/42							
216 ♀	Restricted to dog 213	P.A.[2]	238	117	1.0	11	892
218 ♂	Restricted to dog 213	P.A. and liver	216	109	1.0	12	1042
214 ♀	Restricted to dog 215	P.A. and liver	266	128	0.93	13	892
217 ♂	Restricted to dog 215	P.A.	221	110	0.99	12	908
213 ♂	Ad lib.	No P.A.	124	42	0.51	9	483
215 ♀	Ad. lib.	No P.A.	157	57	0.57	10	717
3/14/42							
213	Ad lib.	2 doses of P.A.	252	92	1.5	16	1092
215	Ad lib.	2 doses of P.A.	198	74	0.60	13	833
3/21/42							
213		Daily doses of P.A.	226	110	0.95	16	967
215		Daily doses of P.A.	178	87	0.96	14	850

[1] Analytical data are given in "mg. per cent."
[2] P.A. = Calcium Pantothenate.

animals received the supplement plus 4 mg. of pantothenic acid per day. Blood samples were taken and analysed at the time intervals shown in table 3.

Group 4. This group of animals, used in conjunction with other experiments, was placed on the basal ration ad libitum July, 1940. Control animals 76 and 80 were given a daily supplement consisting of 0.5 to 1.0 mg. each of thiamine, ribo-

flavin, and pyridoxine, and 5 to 10 mg. each of nicotinic acid and pantothenic acid. Neither choline nor inositol was included. Dog 112 was given the same supplement minus panto-

TABLE 2

Blood lipids in mongrel puppies with pantothenic acid deficiency. Analytical data are in milligrams per cent.

DATE AND DOG NO.	BASAL DIET	SUPPLEMENT CONTAINED	CHOLESTEROL		RATIO	LIPOID P	TOTAL LIPOIDS
			TOTAL	ESTERS			
2/11/42							
207 ♀	Restricted to dog 206	P.A.	357	166	0.87	18	1250
211 ♀	Restricted to dog 206	P.A. and liver	295	132	0.81	17	1133
201 ♂	Ad lib.	P.A.	256	126	0.97	14	1107
202 ♀	Ad lib.	P.A.	252	120	0.91	15	1160
203 ♂	Ad lib.	P.A.	269	132	1.0	13	1147
204 ♂	Restricted to dog 201	P.A. and liver	306	146	0.91	14	1193
205 ♀	Restricted to dog 203	P.A. and liver	298	155	1.1	14	1127
208 ♂	Restricted to dog 210	P.A.	339	158	0.81	17	1225
209 ♂	Restricted to dog 210	P.A. and liver	339	175	1.1	17	1083
210 ♀	Ad lib.	No P.A.	214	75	0.54	14	834
212 ♂	Ad lib.	No P.A.	219	90	0.70	14	927
3/11/42							
206 ♂	Ad lib.	No P.A.	224	96	0.75	15	1117
3/13/42							
206 ♂	Ad lib.	2 doses of P.A.	297	133	0.81	18	1150
3/21/42							
206 ♂	Ad lib.	Daily doses of P.A.	238	121	1.0	17	942
3/21/42							
211 ♀	Restricted to dog 206	P.A.	258	124	0.93	17	1000

thenic acid. Dogs 103 and 113 were given the same supplement minus pantothenic acid and riboflavin. In February of the following year these dogs were given riboflavin and were thus continued with a lack of only pantothenic acid. Through the

TABLE 3 [1]

Blood lipids in paired-fed growing dogs (6½ months old) with pantothenic acid deficiency. Analytical data are in milligrams per cent.

DATE	CHOLESTEROL		RATIO	LIPOID P	TOTAL LIPOIDS	CHOLESTEROL		RATIO	LIPOID P	TOTAL LIPOIDS
	TOTAL	ESTERS				TOTAL	ESTERS			
	Dog 63					*Dog 65*				
12/17/41	271	173	1.8	..	1072	311	185	1.5	18	1000
1/ 7/42	273	185	2.1	18	930	230	156	2.1	19	1173
1/15/42	260	153	1.4	15	...	240	155	1.8	14	...
1/28/42	282	203	2.6	17	...	270	163	1.5	17	...
2/19/42	196	88	0.8	14	550	239	145	1.5	15	658
3/ 7/42	181	115	1.7	15	893	251	116	0.9	19	1125
3/21/42	155	60	0.6	11	517	198	157	3.8	13	766
3/30/42	160	57	0.6	9	634	254	131	1.1	12	875
	Dog 73					*Dog 71*				
12/17/41	304	195	1.8	..	999	259	176	2.1	18	1100
1/ 7/42	273	185	2.1	18	980	271	173	1.8	17	1167
1/15/42	288	193	2.0	19	...	238	178	3.0	16	...
1/28/42	256	162	1.7	17	...	281	217	3.4	17	...
2/19/42	290	193	2.0	17	790	314	225	2.5	20	900
3/ 7/42	271	185	2.2	20	1100	271	183	2.1	19	1300
3/18/42 [2]	190	129	2.1	15	717	295	181	1.6	18	860

[1] Animals placed on test 12/19/41.

[2] Dogs sacrificed in conjunction with other work.

courtesy of Drs. A. O. Seeler and R. H. Silber we studied these dogs from December, 1941 to the present time. The same depressed blood lipoid picture was observed. Upon administration of pantothenic acid (4 mg. per kilogram per day) to dogs 103 and 113, the blood lipoid level increased as shown in table 4.

TABLE 4

Blood lipids of dogs in pantothenic acid deficiency and receiving various vitamin supplements (see text). Analytical data are in "milligrams per cent."

DATE	DOG NO.	CALCIUM PANTOTHENATE IN SUPPLEMENT	CHOLESTEROL		RATIO	LIPOID P	TOTAL LIPOIDS
			TOTAL	ESTERS			
12/3/41	76	+	193	109	1.3	9	777
	80	+	187	87	0.9	8	600
	103	−	88	44	1.0	4	300
	112	−	76	37	1.0	5	300
	113	−	120	64	1.1	7	503
12/23/41[1]	76	+	203	93	0.9	11	776
	80	+	109	51	0.9	6	470
	103	+	180	72	0.7	10	770
	112	−	68	34	1.0	5	490
	113	+	188	103	1.2	12	867
2/16/42	76	+	157	83	1.1	15	816
	80	+	161	99	1.6	13	750
	103	+	188	133	2.4	17	955
	112	−	90	47	1.1	8	458
	113	+	168	104	1.6	14	800

[1] The administration of calcium pantothenate to dogs numbered 103 and 113 was begun 12/14/41.

DISCUSSION

These results indicate that a deficiency of pantothenic acid in the dog produces a lowering of the blood cholesterol, cholesterol esters, lipoid phosphorus, and total lipoids. The blood levels are rapidly increased by as little as 2 daily doses of the vitamin. Recently Schaeffer, McKibbin and Elvehjem ('42) reported that in their pantothenic acid deficient dogs the blood sugar was usually lowered; the blood chlorides were slightly depressed; non-protein nitrogen was increased, and

serum calcium and inorganic phosphorus remained normal in severely deficient dogs. At post-mortem examination the deficient animals showed fatty livers. In our animals the blood urea appeared normal and the blood sugar was lowered only in dog no. 112. Shortly before collapse, the urea nitrogen rose and the blood sugar fell. In our group of young adult setters, the inorganic phosphorus, chlorides, sodium and potassium were not significantly altered. The striking changes occurred in all of our animals in the blood lipoid picture.

Henderson, McIntire, Waisman and Elvehjem ('42) reported that their excretion studies in the rat indicate a decrease in pantothenic acid requirement as the growth rate decreases. More recently, Unna and Richards ('42) reported a striking decrease with progressing age in the requirement of the rat. In the present study it was not possible to follow the blood changes serially in all the animals, but depressed blood lipoids were noted in pups about 40 to 60 days after the animals were placed on pantothenic acid deficient diets, and collapse rapidly followed. Similar lipoid changes as well as loss of appetite and growth rate developed somewhat more slowly in the young adult setters. Depressed blood lipoids were also observed in the adult mongrels, but it should be noted that the onset of the pantothenic acid deficiency symptoms was distinctly delayed in these animals. In agreement with previous findings in the rat, it would appear that a pantothenic acid deficiency is not as critical in adult dogs as it is in pups.[3]

The studies of Epstein ('31, '32) suggest that the depressed cholesterol-cholesterol ester ratios reported here are indicative of liver damage. Through the courtesy of Dr. William Antopol of the Newark Beth Israel Hospital, who is investigating the pathology of pantothenic acid deficiency, we have the following preliminary report:

Dog no. 210 (female, deficient): almost the entire liver is composed of large vacuolated and granular liver cord cells.[4]

[3] See footnote 2, page 274.

[4] All sections except those designated as stained with sudan III were cut from formalin fixed tissue embedded in paraffin and stained with eosin and hematoxylin.

The sinuses are very narrow and in many places abut against one another. Only a rare area, usually nearer the central vein, is not involved in this process. Some of the liver cells contain 2 nuclei and in places there is a moderate variation in size and pyknocity. The Kupffer cells are difficult to distinguish. The findings are strikingly revealed in frozen sections stained with sudan III. Except for small areas, near the center of the lobules the cells are found to be filled universally with sudanophilic substances.

Dog no. 212 (male, deficient): the vacuoles are finer than those in dog no. 210 and the cytoplasm, in places, is more granular. The extent of the involvement however, is almost as great as that in dog no. 210. The variation in nuclear size and staining is not as evident as in no. 210. Sudan stain shows essentially the same finding as in dog no. 210.

Dog. no. 208 (male, given pantothenic acid): shows areas with numerous larger vacuolated and pale granular cells. These for the most part are localized toward the periphery of the lobules. The remainder of the liver is for the most part composed of smaller cells, with a more eosinophilic cytoplasm which is either non-vacuolated, or contains a moderate number of vacuoles. The sinuses are wider than those observed in dogs nos. 210 and 212 and the Kupffer cells are easily distinguishable. Sudan III stain shows the vacuoles to be filled with globules which stain positively.

Dog no. 209 (male, receiving pantothenic acid and liver): shows only a rare small group of liver cord cells, which contain vacuoles. As a rule these are nearer the periphery of the lobule. The liver cord cells are small and the cytoplasm is eosinophilic, but in some of the cells more intensively staining globules and granules are also present. The sinuses are easily distinguishable as are the Kupffer cells. Many of the latter have a fainter staining cytoplasm than the liver cells proper. The differences between these livers are brought out strikingly by the sudan III stain on frozen sections which shows only rare sudanophilic globules.

Although the most extensive liver changes were found in the pantothenic acid deficient animals, dog no. 208 which received pantothenic acid in addition to the stock supplement still showed considerable fat in the liver, whereas dog no. 209, which received both pantothenic acid and whole dried liver, showed minimal changes. This difference in the control animals suggests the presence of unknown factors in the whole dried beef liver. However, the diminished food consumption makes it necessary to consider the significance of a low protein intake.

SUMMARY

In experiments involving twenty-seven dogs, the animals were divided into three age groups consisting of weanlings, young adults and adults. Pantothenic acid deficiency was more critical in weanlings than in adult dogs. Ten of the dogs were maintained on pantothenic acid deficient diets, and all ten of the animals showed a lowered blood cholesterol, cholesterol ester, lipoid phosphorus and total lipoids. Five of the deficient animals were given doses of the vitamin, and the blood lipoid levels were rapidly increased.

Dogs receiving pantothenic acid and whole dried liver in addition to the stock supplement showed minimal liver damage, whereas dogs receiving only pantothenic acid showed considerable fat in their livers. Pantothenic acid deficient dogs developed extremely fatty livers.

LITERATURE CITED

BLOOR, W. R. 1916 The determination of cholesterol in the blood. J. Biol. Chem., vol. 24, p. 227.

BLOOR, W. R., AND ARTHUR KNUDSON 1916 The separate determination of cholesterol and cholesterol esters in small amounts of blood. J. Biol. Chem., vol. 27, p. 107.

EPSTEIN, E. Z. 1931 The cholesterol partition of the blood plasma in parenchymatous diseases of the liver. Arch. Int. Med., vol. 47, p. 82.

———— 1932 Cholesterol of the blood plasma in hepatic and biliary diseases. Arch. Int. Med., vol. 50, p. 203.

HENDERSON, L. M., J. M. MCINTIRE, H. A. WAISMAN AND C. A. ELVEHJEM 1942 Pantothenic acid in the nutrition of the rat. J. Nutrition, vol. 23, p. 47.

SCHAEFFER, A. E., J. M. MCKIBBIN AND C. A. ELVEHJEM 1942 Pantothenic acid deficiency study in dogs. J. Biol. Chem., vol. 143, p. 321.

YOUNGBURG, G. E., AND M. V. YOUNGBURG 1930 Phosphorus metabolism: system of blood phosphorus analysis. J. Lab. Clin. Med., vol. 16, p. 158.

UNNA, K., AND G. V. RICHARDS 1942 Relationship between pantothenic acid requirement and age in the rat. J. Nutrition. (In press.)

THE INTERRELATIONSHIP OF CALCIUM, PHOSPHORUS AND NITROGEN IN THE METABOLISM OF PRE-SCHOOL CHILDREN [1]

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Calcium, phosphorus and nitrogen storage represent only a part of the complicated processes which occur during the growth of children. In all probability, there is an interrelationship between the quantity of any one of these minerals deposited and the amount of the others utilized. Mineral deposition in bone could well be regulated by the availability of either calcium or phosphorus. Phosphorus also forms a part of the molecule of certain proteins and if the quantity available for use in the body were limited, this might reduce nitrogen storage and thus curtail muscle growth. Although growth studies indicate that bone takes precedence over muscle tissue growth, it is possible that under some circumstances a large utilization of phosphorus in soft tissue might reduce the amount available for bone production.

In previous publications, Hawks et al. ('37, '38 and '40) reported studies showing the effect of increasing the protein content of the diet on the protein and calorie utilization of pre-school children. At the same time they also determined calcium and phosphorus balances. This paper discusses the

[1] Published from Michigan State College Agricultural Experiment Station as paper no. 571, new series.

interrelationships between the retentions of calcium, phosphorus and nitrogen.

PROCEDURE

The method of procedure has been discussed in detail (Hawks et al., '37) and therefore only a brief account will be presented here. Two groups of pre-school children received, first, a diet containing 3 and, then, one containing 4 gm. of protein per kilogram of body weight. In the first experiment, the 4-gm. protein diet contained additional milk, part of it skimmed to adjust the calorie value, meat and whole egg, while in the second experiment, the diet had added egg white and gelatin and a reduced quantity of butter. Experimental periods continued for from 15 to 24 days. In both experiments the conditions were carefully controlled. In the first, there was a slight, but probably insignificant, increase in intake of calories, calcium and phosphorus, but in the second, these substances all remained the same on the two diets. The children received adequate quantities of vitamin D daily.[2]

Calcium was determined according to the volumetric procedure of Kramer and Howland ('26). Phosphorus was determined by the uranium titration method of Pincus as outlined by Peters and Van Slyke ('32). Nitrogen was estimated by the official Kjeldahl-Gunning method (Association of Official Agricultural Chemists, '25).

RESULTS

On account of the different method of increasing the protein content of the diet in the two experiments, the results will be discussed separately. They cannot be compared directly with the results of other investigators since the purpose and technic employed were not entirely the same. In order to make direct comparisons, part of the nitrogen values which were previously published (Hawks, Bray and Dye, '38) have

[2] Subjects B and D received 800 and 840 units, respectively, per day, and children V, C and J each had 1700 units per day. These amounts comply with the allowance which the National Research Council's Committee on Foods and Nutrition recently recommended.

been included in the tables with the figures for calcium and phosphorus.

Experiment 1

Diet. Although calcium and phosphorus intakes were higher on the 4- than on the 3-gm. protein diet (table 1), all of them

TABLE 1

Calcium, phosphorus and nitrogen metabolism data expressed per kilogram of body weight.

Experiment I.

MINERAL		CHILD	3-GM. PROTEIN DIET			4-GM. PROTEIN DIET		
			Range	Mean	Proportion of intake [1]	Range	Mean	Proportion of intake [1]
			mg.	*mg.*	%	*mg.*	*mg.*	%
Calcium	Intake	B	58–61	60	....	69–71	70	....
		D	57–60	59	....	68–70	69	....
	Feces	B	50–59	53	88.3	60–61	61	87.4
		D	46–56	49	82.8	53–57	56	81.1
	Urine	B	2–4	2	4.2	2–3	3	3.8
		D	5–6	5	9.3	6–7	7	9.8
	Retention	B	(–2)–8	5	7.5	5–8	6	8.8
		D	(–2)–8	5	7.9	5–8	6	9.1
Phosphorus	Intake	B	64–68	66	....	81–86	84	....
		D	63–67	66	....	81–85	83	....
	Feces	B	21–24	22	34.0	23–30	27	32.6
		D	15–20	18	27.7	21–27	23	27.7
	Urine	B	39–43	41	62.1	49–54	51	60.6
		D	41–46	45	67.9	52–56	54	64.7
	Retention	B	1–6	3	3.9	1–7	6	6.8
		D	1–5	3	4.4	6–7	6	7.6
Nitrogen	Intake	B	500–533	513	....	668–707	693	....
		D	492–526	508	....	662–699	687	....
	Feces	B	63–103	72	14.0	59–105	88	12.8
		D	46–64	53	10.4	57–67	63	9.1
	Urine	B	379–418	403	78.4	503–587	543	78.4
		D	412–444	427	84.2	542–589	572	83.2
	Retention	B	25–54	38	7.6	36–103	62	8.8
		D	18–39	28	5.4	35–90	52	7.7

[1] Percentage calculations were based on total daily figures in order to eliminate errors caused by rounding out the kilogram figures.

fulfilled the generally accepted allowances which Sherman ('41) gives. For calcium the increase amounted to 10 mg. and for phosphorus to 17 and 18 mg. per kilogram of body weight for the two children. Fluctuations in diet values from period to period were extremely small, the coefficients of variation being 2.2 and 2.1% for calcium and phosphorus, respectively, on the 3-gm. protein diet, and 1.3 and 2.3% for the same minerals on the 4-gm. protein diet. The increase in nitrogen intake values on the 4-gm. protein diet was 179 and 180 mg. per kilogram. The figures varied slightly from period to period, the coefficients of variation being 2.3 and 2.0% on the 3- and 4-gm. protein diets, respectively.

Fecal excretion and absorption. The data in this experiment show that with increased dietary calcium, phosphorus and nitrogen the apparent absorption of all three elements was greater. The increase in dietary protein may have produced these higher absorption values since the percentages of the intake values absorbed were higher on the 4- than on the 3-gm. protein diet, with the exception of phosphorus for child D which remained unchanged. Fecal calcium, phosphorus and nitrogen values per kilogram increased with the higher protein diet, but these increases were not as great as those in the diet and thus produced a lower proportional fecal output.

Urinary excretion and retention. The change in the diet caused increases in the retentions of calcium, phosphorus and nitrogen since these values were higher for both children and represented a larger proportion of the intake figures. The increase in retention of calcium was no greater than the increase in absorption, since both apparent absorption and retention values increased on an average 1.3% of the intake. When more calcium was absorbed the body apparently utilized the extra amount available. Although the milligrams of urinary phosphorus increased, they represented a smaller proportion of the phosphorus intake. Thus the high protein diet was associated with an increased phosphorus retention in contrast to the unchanged calcium retentions. The urinary

nitrogen increased on the 4-gm. protein diet but the proportion of the intake eliminated in this manner was approximately the same on the two diets. Therefore the nitrogen retention increased in the same proportion as did the absorption values.

Experiment 2

Diet. In this experiment adjustments made in changing from the 3- to the 4-gm. protein level were such as to eliminate variation in other dietary constituents, and any changes which occurred in calcium and phosphorus metabolism were probably due to the increase in the nitrogen content of the diet. Table 2 shows that, with the exception of the calcium values for subject V, calcium and phosphorus intakes increased only 1 mg. per kilogram with the change to the higher protein diet. The intake values for calcium and phosphorus were similar to, and the figures for nitrogen were slightly lower than, the corresponding figures for the 3-gm. protein diet of the earlier study. Fluctuations from period to period were similar, the coefficients of variation on the two diets being 4.0 and 3.4% for calcium, 3.6 and 3.4% for phosphorus and 3.8 and 3.2% for nitrogen.

Fecal excretion and absorption. In order to make the results comparable with those published in the preceding papers on nitrogen (Hawks, Bray and Dye, '38) and calorie metabolism (Hawks, Voorhees, Bray and Dye, '40), the first 9 days on the 4-gm. protein diet were considered as a preliminary period, and only the values for periods 4 to 8 will be included in the following discussion. The mean values for calcium and phosphorus for all periods and for periods 4 to 8 however, were practically the same.

The data suggest that calcium intake rather than changes in protein content affected calcium absorption. The fecal output for two children increased by the same amount as did the diet values, being 3 mg. per kilogram for subject V and 1 mg. per kilogram for child J. Fecal values for subject C did not change. Absorption figures, therefore, remained almost the same on the 3- as on the 4-gm. protein diet and represented practically the same percentage of the intake values.

TABLE 2

Calcium, phosphorus and nitrogen metabolism data expressed per kilogram of body weight.

Experiment II.

MINERAL		CHILD	3-GM. PROTEIN DIET			4-GM. PROTEIN DIET			
			Range	Mean	Proportion of intake [1]	Range	Mean total	Periods 4–8	Proportion of intake [1]
			mg.	*mg.*	%	*mg.*	*mg.*	*mg.*	%
Calcium	Intake	V	55–58	56	....	57–61	59	59	....
		C	57–61	59	....	58–63	60	60	....
		J	53–57	55	....	55–59	57	56	....
	Feces	V	42–49	46	81.5	46–52	49	49	83.2
		C	45–49	47	80.0	43–54	47	47	77.5
		J	43–49	46	83.8	43–48	46	47	82.9
	Urine	V	2–2	2	3.2	2–3	3	3	4.8
		C	1–2	2	2.6	2–3	2	2	3.6
		J	2–3	3	4.6	3–5	4	3	5.9
	Retention	V	6–11	8	15.3	4–10	7	7	12.0
		C	8–14	10	17.4	7–16	11	11	18.9
		J	4–9	6	11.6	4–10	7	6	11.2
Phosphorus	Intake	V	63–66	65	....	64–68	66	66	....
		C	66–69	67	....	66–69	68	68	....
		J	61–65	63	....	62–65	63	64	....
	Feces	V	21–23	22	34.0	21–26	23	24	36.4
		C	20–22	21	30.9	19–25	22	22	32.7
		J	20–25	23	37.2	22–27	24	25	38.7
	Urine	V	37–39	38	58.1	35–38	37	37	55.4
		C	38–42	40	60.4	36–40	38	38	55.5
		J	33–36	35	55.1	32–36	33	33	51.3
	Retention	V	4–6	5	7.9	2–8	6	5	8.2
		C	3–10	6	8.7	6–10	8	8	11.8
		J	2–8	5	7.7	4–7	6	6	10.0
Nitrogen	Intake	V	436–466	450	....	603–627	618	617	....
		C	452–488	472	....	619–643	635	632	....
		J	423–456	442	....	579–602	595	591	....
	Feces	V	32–35	33	7.3	36–48	41	43	7.0
		C	44–46	45	9.6	41–56	49	50	7.9
		J	52–59	56	12.5	56–61	59	59	9.9
	Urine	V	374–402	392	87.2	508–552	539	542	87.8
		C	379–403	396	83.9	503–641	542	546	86.3
		J	342–374	361	81.7	480–523	497	493	83.4
	Retention	V	13–27	25	5.5	23–78	38	32	5.2
		C	14–47	31	6.5	27–84	44	36	5.8
		J	10–34	25	5.8	16–64	39	39	6.7

[1] Percentage calculations were based on total daily figures in order to eliminate errors caused by rounding out the kilogram figures.

Although the actual changes in phosphorus values were very small, the 4-gm. protein diet slightly reduced the amount absorbed and thus reduced the amount of phosphorus available for use in the body. This was due to the fact that fecal outputs were 1 or 2 mg. per kilogram greater for each child and these higher values represented a larger percentage of the intake on the 4- than on the 3-gm. protein diet.

The increase in the protein content of the diet caused greater nitrogen absorption which represented no increase in the percentage of the intake for subject V but an increase of 2% of the intake for the other two children. The milligrams of nitrogen excreted in the feces increased for all children.

Urinary excretion and retention. The additional protein in the diet apparently did not affect the utilization of absorbed calcium. Although the average urine values per kilogram increased very slightly on the higher protein diet, the total values per day increased by a fairly large proportion of the urinary excretion. Since fecal values represented practically all of the calcium excreted, they obscured any changes in urine figures and made the retentions exactly the same on the two diets.

Although phosphorus retentions were only slightly higher on the 4- than on the 3-gm. protein diet, they represented a larger proportion of the intake values for each child and indicated that increased nitrogen produced a better utilization of phosphorus. On the 4-gm. protein diet the urinary phosphorus decreased more than the absorption figures for all except child V. The actual retentions in the body increased from 6 to 8 mg. per kilogram for subject C and from 5 to 6 mg. per kilogram for child J.

The higher protein diet caused an increase in the milligrams of nitrogen retained by all children. The percentage of the intake retained, however, was practically the same on the two diets.

DISCUSSION

In both experiments, the differences in retention expressed in terms of retention ratios indicated that the metabolic

processes were not the same on the two levels of protein intake (table 3). The results of the two experiments showed that increased protein either from meat, milk and eggs or from egg white and gelatin produced similar changes in tissue growth.

TABLE 3

Retention ratios.[1]

EXPERIMENT	PROTEIN IN DIET	CHILD	Ca : P	N : Ca	N : P	N : P — $\frac{Ca}{2.19}$ [2]
	gm.					
1	3	B	1.7 : 1	8.7 : 1	15.1 : 1	74 : 1
		D	1.6 : 1	6.0 : 1	9.7 : 1	37 : 1
		Mean	1.7 : 1	7.3 : 1	12.4 : 1	56 : 1
	4	B	1.1 : 1	10.0 : 1	10.8 : 1	21 : 1
		D	1.0 : 1	8.4 : 1	8.3 : 1	15 : 1
		Mean	1.0 : 1	9.2 : 1	9.5 : 1	18 : 1
2	3	V	1.7 : 1	2.9 : 1	4.8 : 1	21 : 1
		C	1.7 : 1	3.0 : 1	5.3 : 1	27 : 1
		J	1.3 : 1	4.0 : 1	5.3 : 1	13 : 1
		Mean	1.6 : 1	3.3 : 1	5.1 : 1	20 : 1
	4	V	1.3 : 1	4.6 : 1	5.9 : 1	14 : 1
		C	1.4 : 1	3.2 : 1	4.6 : 1	13 : 1
		J	1.0 : 1	6.2 : 1	6.3 : 1	11 : 1
		Mean	1.2 : 1	4.7 : 1	5.6 : 1	13 : 1

[1] On account of the small figures per kilogram of body weight and the errors involved, these ratios are calculated using the average daily retention values.

[2] $\frac{Ca}{2.19} = \frac{98\% \text{ calcium}}{2.15}$

The Ca : P retention ratios indicated that there was a difference in the relative growth of bone and soft tissue on the 3- and 4-gm. protein diets. From calculations based on chemical analyses of bone, muscle and other tissue, Stearns ('31) suggested that for infants a Ca : P retention ratio of from 1.5 : 1 to 1.6 : 1 represented the average relationship between bone and muscle tissue growth. A lower ratio indicated increased muscular development and a higher ratio indicated a greater growth of bone than muscle tissue. On the 3-gm. protein diet,

all children, with the exception of subject J, were probably producing a normal proportion of bone and muscle tissue since the retention ratios were either 1.6:1 or 1.7:1. With the additional protein in the diet, these ratios were reduced, ranging from 1.0:1 to 1.4:1. This showed an acceleration in the growth of soft tissue as compared to that of bone. Although subject J was apparently producing an excess of soft tissue growth on the 3-gm. protein diet, she, too, had an acceleration in the growth of that same type of tissue on the 4-gm. protein diet, because her retention ratio fell from 1.3:1 to 1.0:1.

Although all children grew at a faster rate on the 4- than on the 3-gm. protein diet (Hawks et al., '37, '38, and '40) the composition of the weight gains was probably not entirely the same for all children on the same diet nor for each child on the two different diets. If the increased weight gains had caused the acceleration of growth of bone tissue there should have been an increase in calcium retention, since the production of bones and teeth utilize from 97 to 98% of the absorbed calcium and the growth of other tissues requires only small amounts of this mineral. For both children in the first study and for child C in the second study, the higher protein diet did increase calcium retentions to a slight degree and thus these children may have had some acceleration of bone growth. For children V and J calcium retention did not increase and, therefore, the high protein diet probably had little if any effect on the bone growth of these children. Since the increase in bone growth on the high protein diet was probably small, soft tissue must have composed a large part of the increased weight gains. The higher nitrogen retentions on the 4-gm. protein diet substantiated this fact. Water storage may have produced some of the extra weight gains, because there was an increase in sodium and chlorine retentions on the higher protein diet and these minerals are associated with fluid tissues in the body (Hawks, Bray, Hartt, Whittemore and Dye, in press). Furthermore fat deposits

may have caused part of the increased weight gains and some phosphorus may have been deposited with this tissue.

The relative growth of bone and protein tissue can also be expressed by a ratio between nitrogen and calcium retentions provided one assumes that all of the nitrogen is utilized in production of protein tissue, and all of the calcium in bony tissue. The change to the 4-gm. protein diet caused an increase in the N : Ca retention ratios for all children, indicating that nitrogen storage increased to a greater extent than that of calcium, and that in all probability protein tissue increased at a faster rate than did bone tissue. In the first study the ratios changed from a mean of 7.3 : 1 on the 3- to a mean of 9.2 : 1 on the 4-gm. protein diet, and in the second study the corresponding changes were from 3.3 : 1 to 4.7 : 1 (table 3).

The N : P retention ratios gave further evidence that all five children were not producing the same type of body tissue. The lower ratios on the higher protein diet showed increased retentions of phosphorus in relation to nitrogen for children B, D and C. This may be explained by the fact that additional phosphorus was probably deposited with the extra calcium which these children stored. There was an increase in the ratios for subjects V and J who did not store additional calcium.

It would be possible to signify differences in the type of soft tissue growth by a ratio between nitrogen and the amount of phosphorus used in excess of that deposited in bones. Since bone contains 2.15 parts of calcium to 1 part of phosphorus (Shohl, '39), it is possible to estimate the amount of phosphorus deposited in the skeleton if one assumes that 98% of the calcium is deposited therein (Stearns, '31). The amount of phosphorus retained in excess of that required for bone growth may then be expressed by the relation, P retained in soft tissues = total P retained $- \frac{98\% \text{ of the calcium}}{2.15}$. This retention ratio then becomes $N : P - \frac{Ca}{2.19}$.

The ratios between nitrogen and this new value for phosphorus indicated that on the lower protein diet the children were producing tissues which did not have a composition

similar to that of body tissue as a whole. With the exception of child J, the ratios were all above 17 : 1 which Stearns ('31) said approximated that for all body tissue other than bone. This suggests that the children were at that time manufacturing tissue of some particular type high in nitrogen. In blood, for example, the ratio is 53 : 1. It is also possible that some of that nitrogen may have been used to build up a reserve in the body and that all of it may not have been laid down in conjunction with phosphorus in muscle tissue.

The reduction of these ratios on the high protein diet may indicate that muscle was the dominant type of tissue manufactured, since it has an approximate N : non-osseous P ratio of 15 : 1. Calcium utilization was practically the same on the two diets, thus nitrogen intake probably had little influence on bone growth.

SUMMARY

1. Five pre-school children received, during 15- to 24-day periods, diets containing first, 3 and then 4 gm. of protein per kilogram of body weight.

2. For two children whole egg, meat and milk increased the nitrogen content of the diet while for the others egg white and gelatin were used.

3. The higher protein diet had no effect on calcium absorption or retention. It caused a decrease in the absorption of phosphorus but there was a better utilization of the amount available. There was an increase in both nitrogen absorption and retention.

4. Weight gains were greater on the 4- than on the 3-gm. protein diet and represented different types of tissue growth. Decided increases in nitrogen retentions and significant changes in retention ratios for Ca : P, N : Ca, N : total P and N : non-osseous P, suggested that muscle and other soft tissue accounted for the greater proportion of the increased weight gains. There may have been additional bone growth in three children who had increased calcium retentions.

LITERATURE CITED

ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS 1925 Official and tentative methods of analysis. The Association, Washington, D. C.

HAWKS, J. E., M. DYE AND M. M. BRAY 1937 An improved technic for metabolism studies in pre-school children with a statistical determination of its reliability. J. Nutrition, vol. 13, p. 51.

HAWKS, J. E., M. M. BRAY AND M. DYE 1938 The influence of diet on the nitrogen balances of pre-school children. J. Nutrition, vol. 15, p. 125.

HAWKS, J. E., J. M. VOORHEES, M. M. BRAY AND M. DYE 1940 The influence of the nitrogen content of the diet on the calorie balances of pre-school children. J. Nutrition, vol. 19, p. 77.

HAWKS, J. E., M. M. BRAY, S. HARTT, M. B. WHITTEMORE AND M. DYE Potassium, sodium and chlorine balances of pre-school children receiving medium and high protein diets. (In press.)

KRAMER, B., AND J. HOWLAND 1926 The quantitative estimation of calcium, magnesium, phosphate, and carbonate in bone. J. Biol. Chem., vol. 68, p. 711.

PETERS, J. P., AND D. D. VAN SLYKE 1932 Quantitative clinical chemistry. Vol. II, Methods, p. 850. The Williams and Wilkins Co., Baltimore.

SHOHL, A. T. 1939 Mineral metabolism. Am. Chem. Soc. Monograph No. 82. Reinhold Publishing Corp.

SHERMAN, H. C. 1941 Chemistry of food and nutrition, 6th edition. The Macmillan Company, New York.

STEARNS, G. 1931 The significance of the retention ratio of calcium: phosphorus in infants and in children. Am. J. Dis. Child., vol. 42, p. 749.

THE EFFECT OF THE LEVEL OF PROTEIN INTAKE UPON THE URINARY EXCRETION OF RIBOFLAVIN AND NICOTINIC ACID IN DOGS AND RATS [1]

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TWO FIGURES

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There is evidence to indicate a relationship between protein metabolism and the vitamins of the B-complex. An increase in the protein content of the diet without a corresponding increase in B-complex supplement results in poor growth of rats (Hartwell, '25; Reader and Drummond, '25). The development of a B-avitaminosis in rats is accelerated by diets high in protein (Tscherkes, '26). In self-selection feeding experiments rats eat more protein when given the B-vitamins (Richter and Barelare, '39). The dermatitis produced by a deficiency of pyridoxine occurs more readily on a diet containing 30% casein than on a diet containing lower levels of protein (Conger and Elvehjem, '41). Retention of protein by chicks on diets deficient in riboflavin is less than that of pair-fed controls (Kleiber and Jukes, '42). In growing rats riboflavin was found to have a profound effect on the utilization of food for the synthesis of tissue fat and protein (Sure and Dichek, '41).

[1] A preliminary report was given before the American Society of Biological Chemists, April, 1942 (Federation Proceedings I, 132, 1942). Aided in part by the John and Mary R. Markle Foundation, the International Health Division of the Rockefeller Foundation and the Duke University Research Council.

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In the present work, the relationship of protein metabolism to the vitamins, riboflavin and nicotinic acid, was studied in dogs and rats by measuring the urinary excretion of the vitamins as a function of protein intake. In dogs the excretion of thiamine and pyridoxine was also measured.

MATERIALS AND METHODS

Young and adult dogs, maintained in individual cages, and young and adult rats, maintained in groups of four to six, were fed diets containing mixtures of purified casein,[3] beef suet, cornstarch, sucrose, salt mixture, cod liver oil, and dried brewer's yeast.[4] The fats and a suspension of casein and yeast were added to a hot mixture of salts, sucrose, and starch that had been cooked about 45 minutes. The final mixture was cooled and kept in the refrigerator. The diets were to provide three different levels of protein, designed and fed so as to provide approximately the same caloric and vitamin intake per unit of body weight. Each dog was given daily 175 gm. (160 cal.) of cooked food per kilo of body weight. Compensating changes in carbohydrate or fat, or both, were made for changes in protein intake. The composition of the diets used is given in table 1. The low protein diet contained 2.6%, the medium protein diet 17.6% and the high protein diet 41% of the total calories as protein.

Each animal, or group, was maintained at least once for a period of 1 to 2 weeks on each diet, and in the case of the dogs, was given a test dose of riboflavin, nicotinic acid, pyridoxine, and thiamine at the end of each period. The daily food intake was measured, and the animals were weighed every second day.

Urines were collected daily in dark bottles containing toluene and sufficient hydrochloric acid to give a final pH of about 2. Analyses were made on daily or combined samples of urine. Riboflavin was determined fluorometrically (Ferrebee, '40), total nicotinic acid (trigonelline and acid-hydro-

[3] Labco.

[4] Fleischmann.

lyzable derivatives) chemically (Perlzweig, Levy and Sarett, '40), thiamine by its effect on yeast fermentation (Atkin, Schultz and Frey, '39), and nitrogen by nesslerization after digestion with sulfuric acid and hydrogen peroxide. The method employed to measure pyridoxine (Scudi, Buhs and Hood, '42; Bird, Vandenbelt and Emmett, '42) permitted only the analysis of the response to the test dose.

The details of experiments that differ from those outlined above are given in the appropriate place.

TABLE 1

Composition of experimental diets of varying protein content.

INGREDIENT	FOR DOGS			FOR RATS		
	Low	Medium	High	Low	Medium	High
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Purified casein (Labco)	1	18	45	1	18	45
Cornstarch	40	40	40	59	60	60
Sucrose	19(60)[1]	27 (2)[1]	20	..	7	..
Beef suet	21 (2)[1]	10(21)[1]	1	21	10	1
Cod liver oil	2	2	2	2	2	2
B.D.H. salt mixture	3	3	3	3	3	3
Dried brewer's yeast [2] (Fleischmann)	4.5	4.5	4.5	4	4	4

Water added to give 500 gm. of cooked diet.

[1] Fat and carbohydrate contents of the diets were reversed in some experiments to show that the effects obtained were not due to the particular concentrations of these substances.

[2] The yeast contained about 25 μg. of thiamine, 50 μg. of riboflavin and 520 μg. of nicotinic acid per gram.

EXPERIMENTAL

Effect of protein intake on the urinary excretion of riboflavin, nicotinic acid, and thiamine. The data obtained on three growing dogs (G, H, and I) and two adult dogs (J and K), are given in table 2. The data show that the excretion of riboflavin and total nicotinic acid was highest during the periods of low protein intake. The increase in protein intake corresponding to the change from the medium to the high

protein diet had practically no effect on the excretion of riboflavin, but decreased the excretion of nicotinic acid slightly. The thiamine excretion during the periods indicated by table 2 was measured, but since there was no correlation with the

TABLE 2

The average daily intake and excretion of nitrogen, riboflavin and nicotinic acid by five dogs on low, medium and high protein diets, and the per cent excretion of test doses of the vitamins after periods on these diets.

DOG	DIET	NO. OF DAYS	CHANGE IN BODY WEIGHT	NITROGEN		RIBOFLAVIN		NICOTINIC ACID		EXCRETION OF TEST DOSE AFTER EACH PERIOD [2]		
				Intake	Urinary excretion	Intake	Urinary excretion	Intake	Urinary excretion [1]	Riboflavin	Nicotinic acid	Pyridoxine
			kg.	*gm.*	*gm.*	*μg.*	*μg.*	*mg.*	*mg.*	%	%	%
G	High	14	+ 0.4	7.1	2.9	209	71	2.2	0.6			
(3.2	Low	11	− 0.2	0.6	0.8	277	401	2.9	1.5	78	35	36
kg.)	Med.	6	+ 0.2	4.1	1.5	287	71	3.0	1.2	14	32	24
	Low	6	− 0.1	0.4	0.7	197	350	2.0	1.5	62	28	31
	High	6	+ 0.3	7.7	3.1	230	85	2.7	0.9	6	35	20
H	Low	14	− 0.1	0.6	0.7	300	212	3.2	2.3			
(4.2	Med.	11	+ 0.5	4.7	1.6	324	64	3.4	1.7	32	50	31
kg.)	Med.	6	+ 0.4	5.6	1.8	387	70	4.1	1.2	32	50	23
	Low	6	+ 0.2	0.9	1.0	428	485	4.4	2.3	68	75	24
	High	6	+ 0.6	10.2	3.2	302	73	3.1	1.9	3	32	20
I	Med.	14	+ 0.2	2.8	1.0	195	64	2.0	0.7			
(2.8	High	11	+ 0.7	7.2	2.2	222	83	2.3	1.0	0	24	13
kg.)	Med.	6	+ 0.2	4.1	1.6	283	65	3.0	1.0	31	47	20
	Low	6	+ 0.1	0.6	0.7	320	405	3.3	1.3	74	51	19
	High	6	+ 0.7	8.5	2.4	250	83	2.6	1.3	3	37	18
J	High	9	+ 0.1	4.8	3.5	147	65	1.5	1.0	31	68	57
(5.9	Low	9	− 0.2	0.4	1.4	334	260	3.5	2.6	75	97	78
kg.)												
K	Med.	6	0	2.2	1.4	164	64	1.7	0.9	32	60	53
(7.1	Low	10	− 0.1	0.2	1.1	312	129	3.2	1.7	63	81	61
kg.)	High	9	+ 0.2	5.2	3.3	160	57	1.7	0.7	29	65	47
	Low	9	− 0.2	0.3	1.3	270	184	2.8	1.4	100	69	..

[1] Nicotinic acid excretion represents total urinary trigonelline and acid hydrolyzable nicotinic acid derivatives.

[2] The test doses were given subcutaneously and contained 220 μg. riboflavin, 2.2 mg. nicotinamide, 2.2 mg. pyridoxine and 110 μg. thiamine per kilo body weight. The figures given represent the per cent of the dose excreted after subtraction of the value for the preceding day.

protein intake the data are not given. The effects of reciprocal changes in carbohydrate and fat content of low and medium protein diets (table 1) were studied during periods not shown in table 2. The changes did not affect the excretion of riboflavin or total nicotinic acid.

Data on dog G are given in figure 1 showing the changes in riboflavin and nicotinic acid excretion during the periods indicated in table 2. The data in figure 1 and similar data on the other dogs indicate that the change in riboflavin excretion

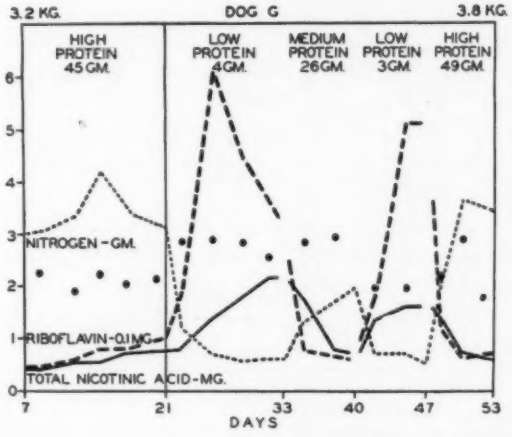

Fig. 1 The urinary excretion of nitrogen, riboflavin, and nicotinic acid by dog G on low, medium and high protein diets. The single black dots represent the riboflavin intake.

produced by the transfer from the medium or high protein diet to the low one, or the converse, was comparatively rapid, and that a maximum excretion on the low diet was reached in 3 to 6 days. The changes in nicotinic acid excretion were slower than those in riboflavin excretion. The excretion of nicotinic acid on the low protein diet continued to increase during 6 to 14 days of those periods, except for the first period of dog H where the excretion was approximately constant from the seventh to the fourteenth day.

As indicated by the data in figure 2 the effect of the protein content of the diet on the urinary excretion of riboflavin by young and adult rats was similar to that obtained in dogs. In rats, however, the increase in protein intake corresponding to the change from the medium to the high protein diet lowered the excretion of riboflavin. The effect of the diets on the excretion of nicotinic acid by rats differed from the effect obtained in dogs. As shown in figure 2 the change from the medium

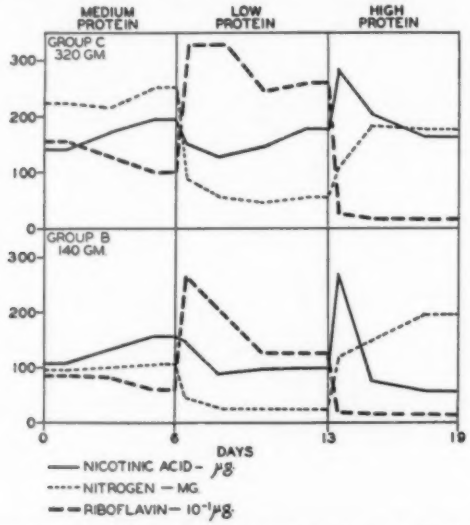

Fig. 2 The urinary excretion of nitrogen, riboflavin, and nicotinic acid per rat, by young and adult male rats on low, medium and high protein diets.

to the low protein diet resulted in an initial decrease in excretion. The change from the low to the high protein diet resulted in an initial increase in excretion which is followed by a decrease. Changes in the excretion of nicotinic acid by rats similar to those shown in figure 2 have been obtained by feeding protein and other nitrogenous compounds (Huff and Perlzweig, '42). The difference between the effect in dogs and in rats may be due to the fact that rats synthesize nicotinic acid (Huff and Perlzweig, '42; Dann, '41).

The low urinary excretion of riboflavin and of nicotinic acid on the medium and high protein diets was associated with an increased excretion of nitrogen, a positive nitrogen balance, and a gain in weight. The high excretion of riboflavin and of nicotinic acid on the low protein diets was associated with a decreased excretion of nitrogen, a negative nitrogen balance, and occasionally with a loss of weight.

The low urinary excretion of the vitamins on the medium and high protein diets may be due either to the catabolism of protein, which leads to the increase in urinary nitrogen, or to the anabolism of protein which leads to the retention of protein nitrogen, or both. The following experiments suggest that the low excretion is not due to the catabolism of protein.

Effect of protein intake on the response to test doses of riboflavin, nicotinic acid, pyridoxine and thiamine. A mixture containing 220 μg. of riboflavin, 2.2 mg. of nicotinamide, 110 μg. of thiamine, and 2.2 mg. of pyridoxine [5] per kilo of body weight was injected into the dogs subcutaneously on the last day of each period indicated in table 2. The differences between the excretions on the preceding day and on the day of the test dose are reported in table 2 as the per cent of the test dose excreted. Since the results of the test dose in the case of thiamine were not affected by the protein intake the data are omitted.

The per cent of the test dose of riboflavin excreted by all dogs was highest on the low protein diet and lowest on the high protein diet. Where a difference in response to the test dose of nicotinic acid was obtained (dogs H, I, J, and K) the per cent of the test dose excreted was highest on the low protein diet. Where a difference in response to the test dose of pyridoxine was obtained (dogs G, J, and K) the per cent of the test dose excreted was also highest on the low protein diet.

If the differences between the responses to the test doses on the various diets represent differences in retention, the data

[5] These synthetic vitamins were generously supplied by Merck and Company, Rahway, New Jersey.

for riboflavin indicate a greater retention as the protein content of the diet is increased. The data for nicotinic acid and pyridoxine suggest a similar relationship between retention and protein intake.

Effect of the replacement of casein by glycine on the urinary excretion of riboflavin and nicotinic acid by dogs. Three young dogs were maintained for several days on the medium protein diet, and then on a similar diet in which the casein was replaced by glycine in terms of nitrogen equivalents. The data obtained on the three dogs were sufficiently uniform to permit consideration of average values. The averages are presented in table 3. The increase in excretion of riboflavin produced by

TABLE 3

The effect of substitution of glycine for casein in the diet of three dogs, L, M and O, upon the urinary excretion of nitrogen, riboflavin and nicotinic acid.

DIET	DAYS	AVERAGE WEIGHT	NITROGEN		RIBOFLAVIN		NICOTINIC ACID	
			Intake	Urinary excretion	Intake	Urinary excretion	Intake	Urinary excretion
		kg.	*gm.*	*gm.*	*γ.*	*γ.*	*mg.*	*mg.*
Medium		2.51	2.8	0.8	190	36	2.1	0.4
Medium diet	1	2.51	3.2	2.8	190	49	2.1	1.3
with glycine	2 + 3	2.54	3.2	2.7	190	69	2.1	1.5
substituted	4	2.54	3.2	2.8	190	119	2.1	2.0
for casein	5	2.52	3.2	2.5	190	165	2.1	1.9
	6	2.48	3.1	2.4	187	146	2.1	2.1

the change from casein to glycine was similar to that obtained in the other dogs by the change from the medium to the low protein diet; however, in the change to glycine the increase in excretion of riboflavin occurred somewhat more slowly and the increase in excretion of nicotinic acid somewhat more rapidly than in the change to the low protein diet.

The fact that the excretion of riboflavin and nicotinic acid increased during a period of active amino acid catabolism suggests that the decrease in excretion of riboflavin and nicotinic acid produced by an increased protein intake (cf. table 2) was not due to the catabolic processes that led to the increase in urinary nitrogen.

Effect of starvation on the urinary excretion of riboflavin and nicotinic acid by dogs. The data in table 4 show that the urinary excretion of riboflavin and nicotinic acid increased during a period of starvation, which represented a period of

TABLE 4

The effect of starvation upon the daily urinary excretion of nitrogen, riboflavin and nicotinic acid by three young dogs.

DIET	DAYS	URINARY NITROGEN			URINARY RIBOFLAVIN			URINARY TOTAL NICOTINIC ACID		
		L 3.2 kg.	M 2.6 kg.	O 3.5 kg.	L	M	O	L	M	O
		gm.	gm.	gm.	μg.	μg.	μg.	mg.	mg.	mg.
Medium		1.0	0.5	1.0	36	25	40	0.6	0.4	0.6
Fasting	1 + 2	1.2	0.7	1.3	43	29	43	2.1	0.9	1.0
	3 + 4	1.4	0.9	1.4	83	48	56	2.3	1.5	1.4
	5 + 6	1.5	0.6	1.3	195	78	116	3.6	1.7	3.8
	7	1.0	0.8	1.3	124	130	250	2.5	1.9	2.8
50–120 gm. of cornstarch and sucrose per day	8	1.1	0.8	0.9	108	105	105	2.6	1.7	1.2
	9 + 10	0.4	0.2	0.3	63	29	52	0.7	0.3	0.4
Medium [1]			1.2	1.6		33	38		0.5	1.1
Fasting plus a vitamin capsule [2]	1 + 2		0.9	1.2		41	38		1.9	5.1
	3 + 4		1.0	1.5		130	120		3.7	3.8
	5 + 6		1.5	1.5		550	460		4.2	3.8
	7 + 8		1.4	1.5		597	525		4.1	2.9

[1] Body weights of M and O at the beginning of this experiment were 3.4 kg. and 4.4 kg., respectively.

[2] Vitamin capsule (Lederle) was a concentrated liver extract and contained 1.5 mg. of nicotinamide and 375 μg. of riboflavin.

protein metabolism that was catabolic.[6] The ingestion of carbohydrate after the period of starvation decreased the catabolism of protein and the excretion of the vitamins. These experiments again suggest that the decrease in excretion of riboflavin and nicotinic acid accompanying an increase in pro-

[6] Similar increases in riboflavin excretion were also found in eleven normal human subjects during 3 to 6-day periods of fasting.

tein intake is due to an anabolic process and is not due to participation in protein catabolism.

Dogs M and O were subjected to another period of fasting a few weeks later, during which each received a daily oral dose of a liver concentrate [7] containing 375 μg. of riboflavin and 1.5 mg. of nicotinamide. The vitamin intake had no obvious effect on the nitrogen excretion. A significant increase in the excretion of riboflavin above that obtained during the first period of fasting when no vitamins were given did not occur until the third or fourth day. An increase in nicotinic acid excretion above that obtained during the first period of fasting was observed as early as the first day. The degree to which the vitamin concentration of the tissues of the animal approaches a maximum level and the efficiency of the mechanisms for conserving each of the vitamins may account for the delay in the excretion of the ingested riboflavin and the prompt appearance of the ingested nicotinic acid. These factors may also determine in part the rate and extent of change in excretion produced by the different diets.

DISCUSSION

The preceding experiments show that an increase in the protein content of the diet, associated with a positive nitrogen balance, produced a decrease in the urinary excretion of nicotinic acid by dogs and of riboflavin by both dogs and rats. The decrease in urinary excretion of the vitamins was probably due, in part, to a retention of the vitamins, since a decrease in the protein content of the diet, associated with a negative nitrogen balance, produced an increase in the urinary excretion of the vitamins. The apparent retention of the vitamins on the high protein diet was probably not due to the increased protein catabolism, since the catabolism during starvation and during the period of glycine ingestion was accompanied by an increased excretion of the vitamins. If the retention of the vitamins during the period of high protein ingestion was not due to protein catabolism, it is conceivable

[7] Lederle.

that the retention was due mainly to protein anabolism. Whether there is actual retention, and whether it is due to protein anabolism, are being investigated by complete balance experiments including tissue analyses. That riboflavin is concerned with protein anabolism is shown (Kleiber and Jukes, '42) by the fact that the protein content of chicks deficient in riboflavin is less than that of pair-fed controls. The experiments described also suggest that a similar relationship may exist between protein anabolism and nicotinic acid and pyridoxine.

SUMMARY

The urinary excretions of nicotinic acid by dogs and of riboflavin by both dogs and rats bear an inverse relationship to the level of protein intake.

Riboflavin and nicotinic acid are inferred to be concerned in protein anabolism.

LITERATURE CITED

ATKIN, L., A. S. SCHULTZ AND C. N. FREY 1939 Ultramicrodetermination of thiamine by the fermentation method. J. Biol. Chem., vol. 129, p. 471.

BIRD, O. D., J. M. VANDENBELT AND A. D. EMMETT 1942 Adaptation of the Scudi colorimetric method for pyridoxine. J. Biol. Chem., vol. 142, p. 317.

CONGER, T. W., AND C. A. ELVEHJEM 1941 The biological estimation of pyridoxine. J. Biol. Chem., vol. 138, p. 555.

DANN, W. J. 1941 The synthesis of nicotinic acid by the rat. J. Biol. Chem., vol. 141, p. 803.

FERREBEE, J. W. 1940 The urinary excretion of riboflavin. Fluorometric methods for its estimation. J. Clin. Invest., vol. 19, p. 251.

HARTWELL, G. A. 1925 Mammary secretion in rats. VI. Vitamin B and the lactating rat's diet. Biochem. J., vol. 19, p. 1075.

HUFF, J. W., AND W. A. PERLZWEIG 1942 Studies in nicotinic acid metabolism. III. Metabolism and synthesis of nicotinic acid in the rat. J. Biol. Chem., vol. 142, p. 401.

KLEIBER, M., AND T. H. JUKES 1942 Metabolism and food utilization of riboflavin-deficient chicks. Proc. Soc. Exp. Biol. Med., vol. 49, p. 34.

PERLZWEIG, W. A., E. D. LEVY AND H. P. SARETT 1940 Nicotinic acid derivatives in human urine and their determination. J. Biol. Chem., vol. 136, p. 729.

READER, V., AND J. C. DRUMMOND 1925 Relation between vitamin B and protein in the diet of growing rats. Biochem. J., vol. 20, p. 1256.

RICHTER, C. P., AND B. BARELARE, JR. 1939 Further observations on the carbohydrate, fat and protein appetite of vitamin B deficient rats. Am. J. Physiol., vol. 127, p. 199.

SCUDI, J. V., R. P. BUHS AND D. B. HOOD 1942 The metabolism of vitamin B_6. J. Biol. Chem., vol. 142, p. 323.

SURE, B., AND M. DICHEK 1941 Riboflavin as a factor in economy of food utilization. J. Nutrition, vol. 21, p. 453.

TSCHERKES, L. A. 1926 Studien über B-avitaminose 1. Biochem. Z., vol. 167, p. 203.

ERRATUM

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In table 2, page 601, the third entry should read:

Caveness et al. ('41)	Mean 138	Mean 62
	stand. dev. 62	stand. dev. 17